



US009447159B2

(12) **United States Patent**  
**Ast et al.**

(10) **Patent No.:** **US 9,447,159 B2**  
(45) **Date of Patent:** **Sep. 20, 2016**

(54) **IMMUNOCONJUGATES**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(21) Appl. No.: **13/457,039**

(22) Filed: **Apr. 26, 2012**

(65) **Prior Publication Data**

US 2012/0276125 A1 Nov. 1, 2012

(30) **Foreign Application Priority Data**

Apr. 29, 2011 (EP) ..... 11164237

(51) **Int. Cl.**

<b>C07K 16/46</b>	(2006.01)
<b>C07K 14/54</b>	(2006.01)
<b>C07K 14/55</b>	(2006.01)
<b>A61K 47/48</b>	(2006.01)
<b>C07K 16/18</b>	(2006.01)
<b>C07K 16/28</b>	(2006.01)
<b>C07K 16/30</b>	(2006.01)
<b>C07K 16/40</b>	(2006.01)
<b>A61K 39/44</b>	(2006.01)
<b>C12N 15/13</b>	(2006.01)
<b>C12N 15/63</b>	(2006.01)
<b>C12N 5/10</b>	(2006.01)
<b>C12P 21/00</b>	(2006.01)

(52) **U.S. Cl.**

CPC ..... **C07K 14/5434** (2013.01); **A61K 47/48423** (2013.01); **A61K 47/48538** (2013.01); **A61K 47/48561** (2013.01); **A61K 47/48569** (2013.01); **A61K 47/48576** (2013.01); **C07K 14/55** (2013.01); **C07K 16/18** (2013.01); **C07K 16/2866** (2013.01); **C07K 16/30** (2013.01); **C07K 16/3007** (2013.01); **C07K 16/40** (2013.01); **C07K 2317/41** (2013.01); **C07K 2317/524** (2013.01); **C07K 2317/55** (2013.01); **C07K 2317/71** (2013.01); **C07K 2317/732** (2013.01); **C07K 2317/92** (2013.01); **C07K 2319/00** (2013.01); **C07K 2319/30** (2013.01); **C07K 2319/33** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention generally relates to antigen-specific immunoconjugates for selectively delivering effector moieties that influence cellular activity. More specifically, the invention provides novel immunoconjugates comprising a first antigen binding moiety, an Fc domain and a single effector moiety. In addition, the present invention relates to polynucleotides encoding such immunoconjugates, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the immunoconjugates of the invention, and to methods of using these immunoconjugates in the treatment of disease.

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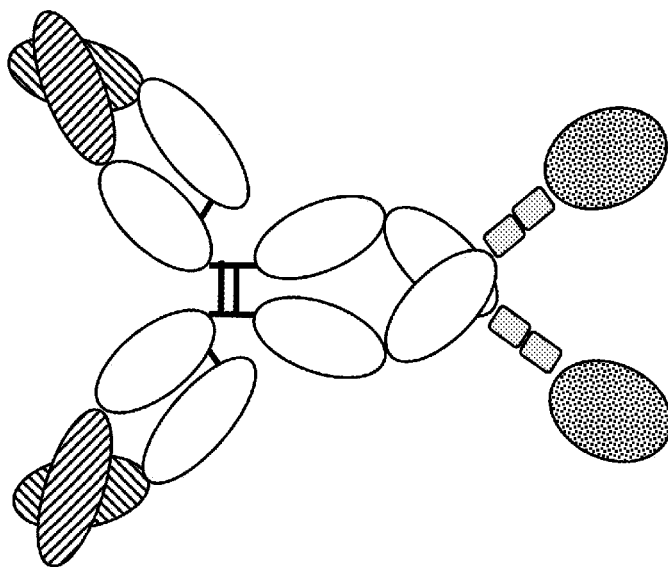
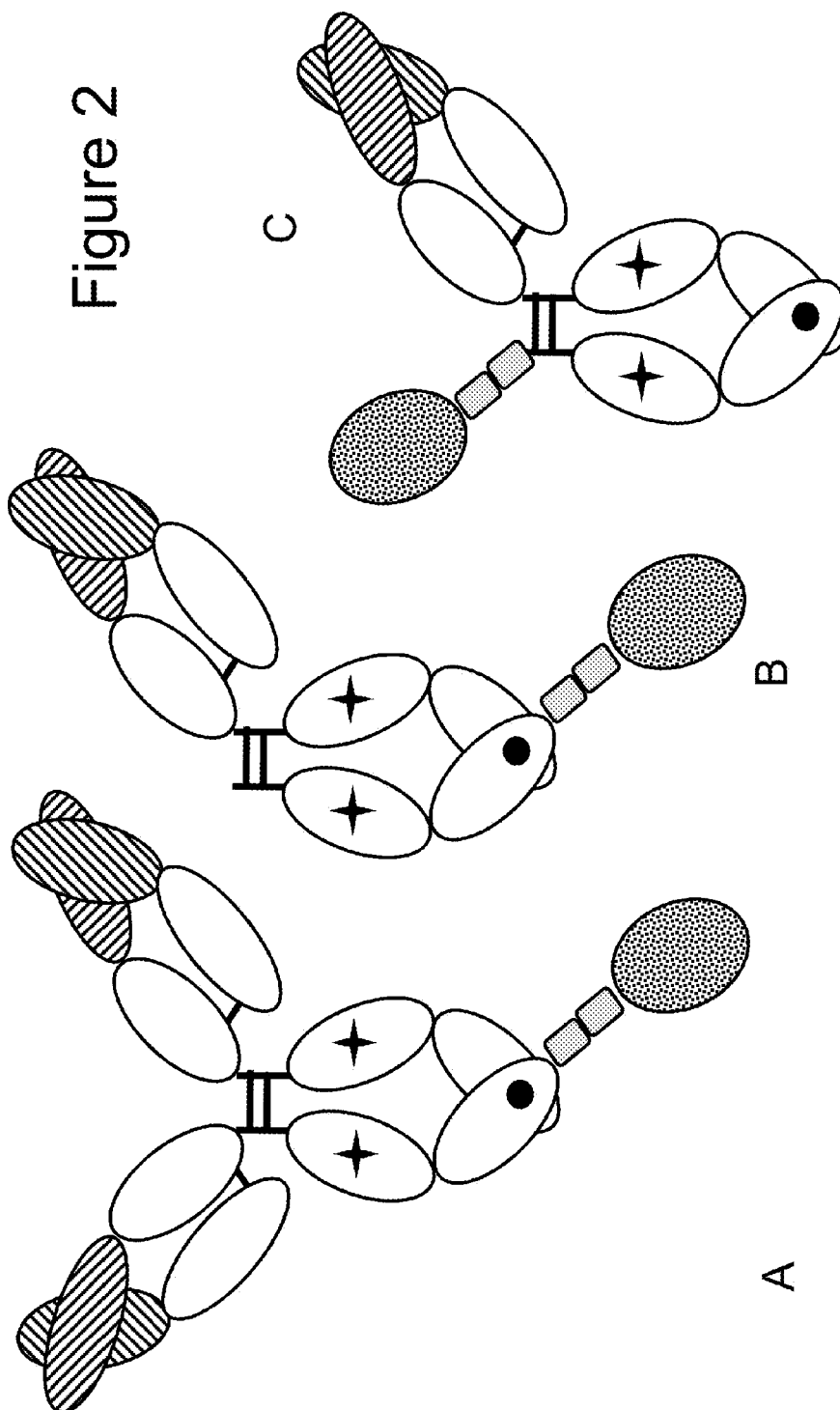
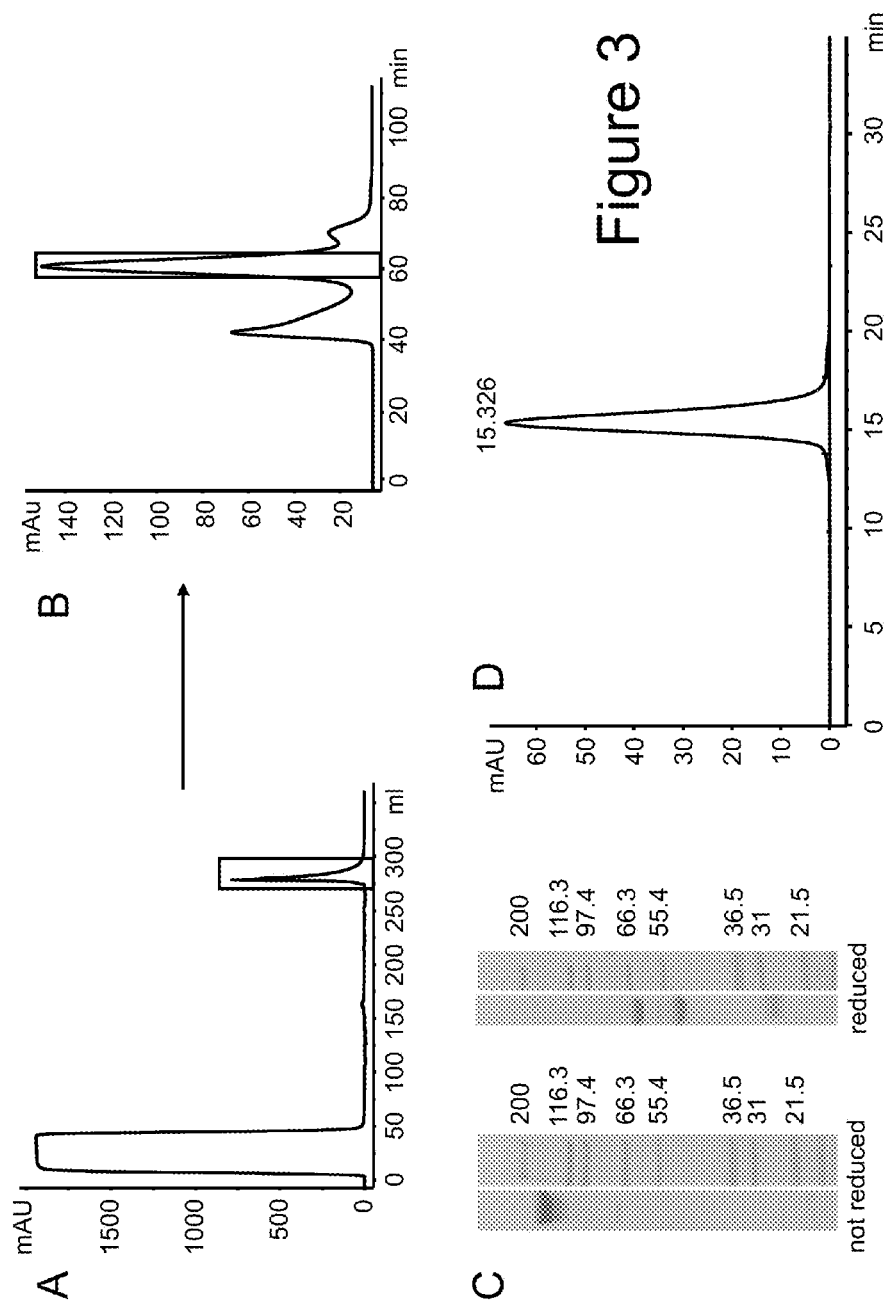
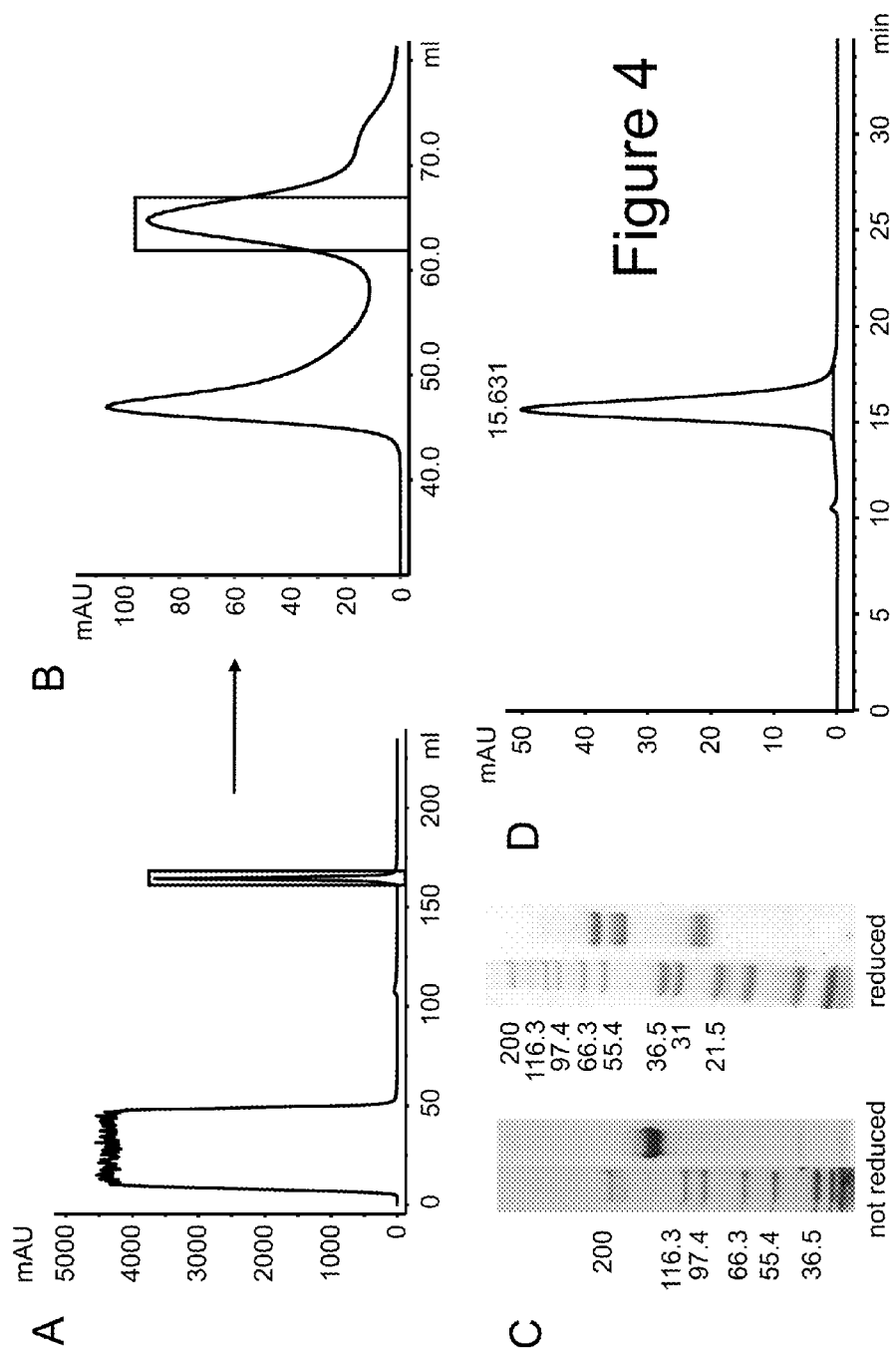


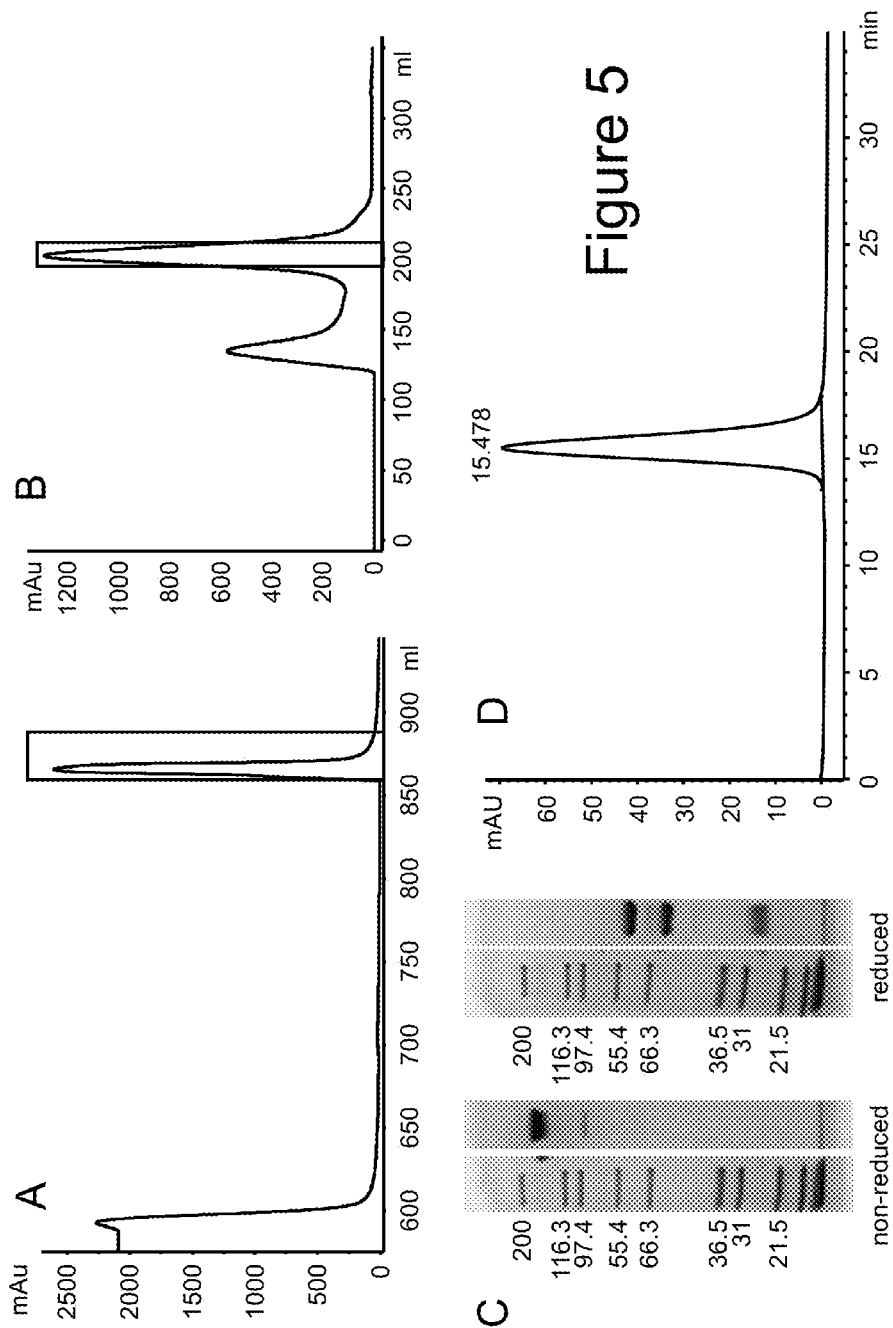
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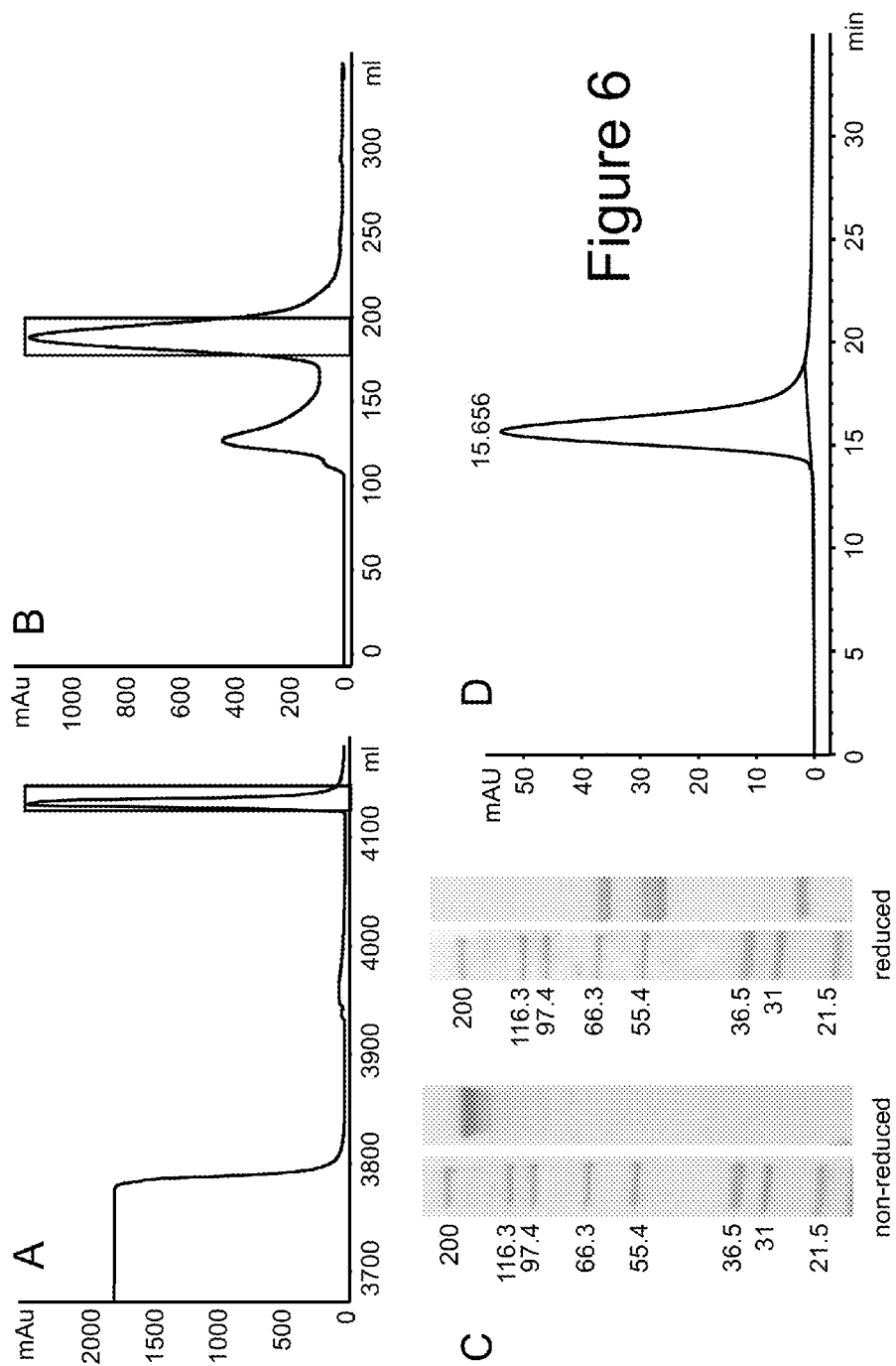
Figure 2











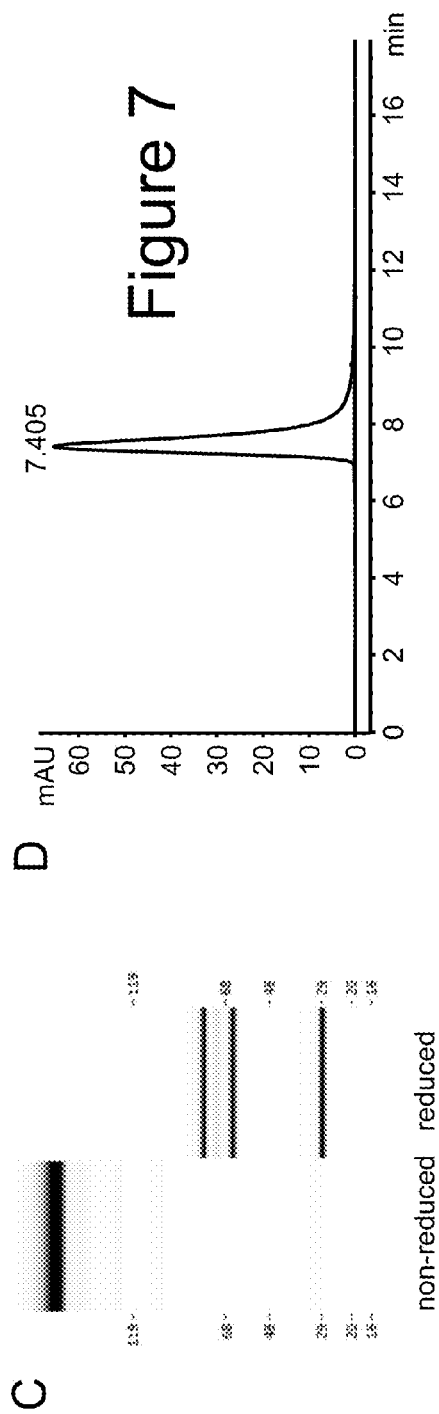
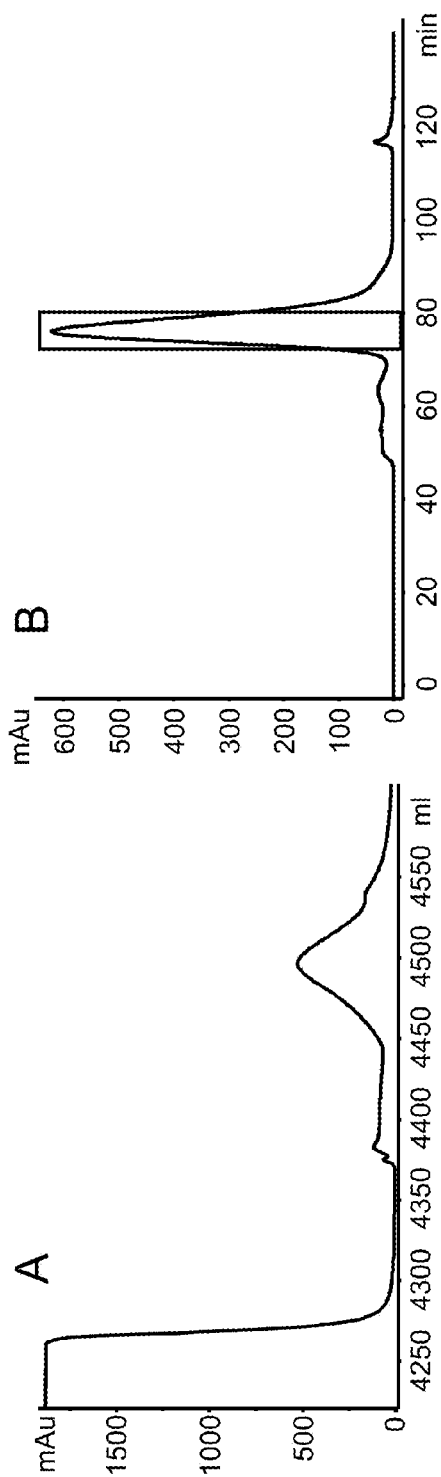
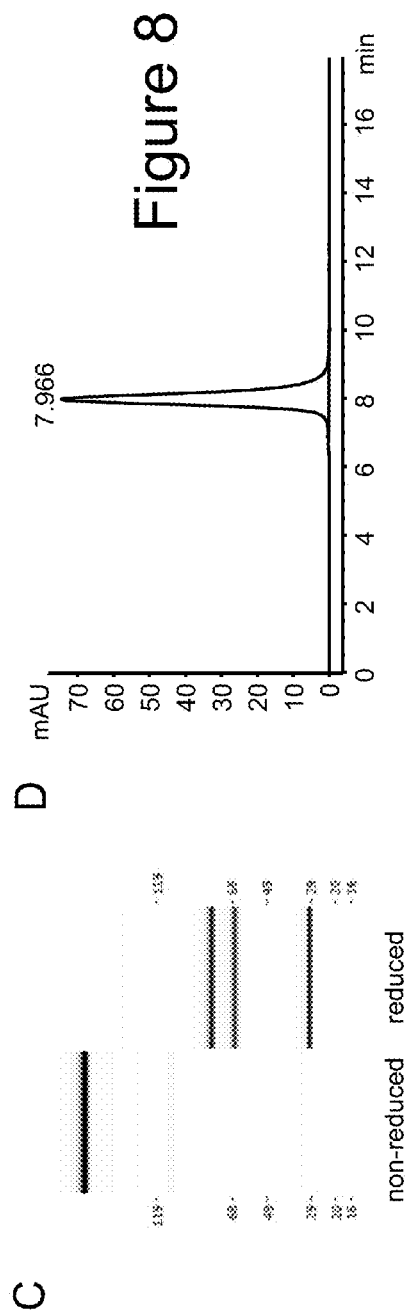
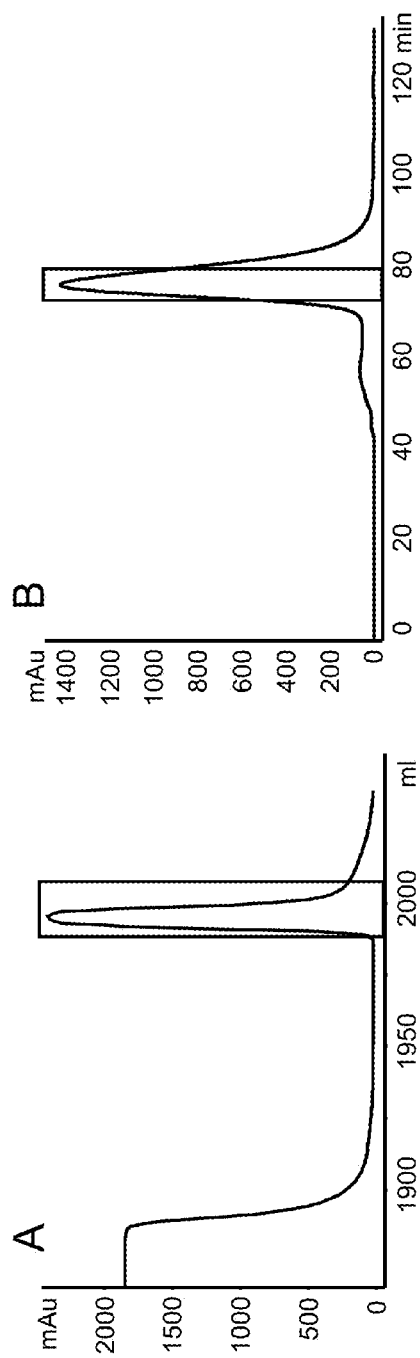


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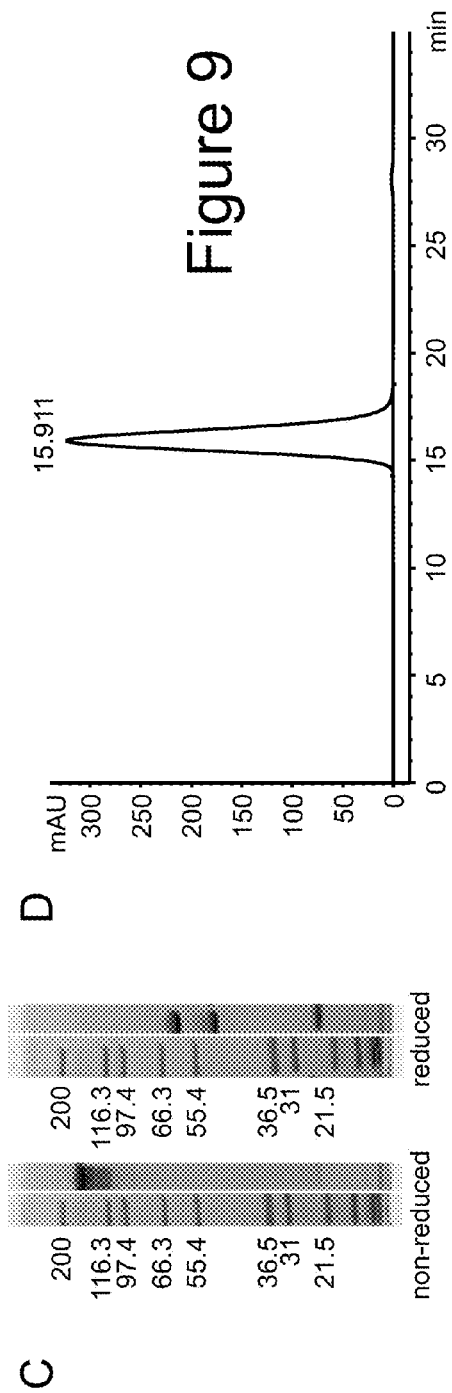
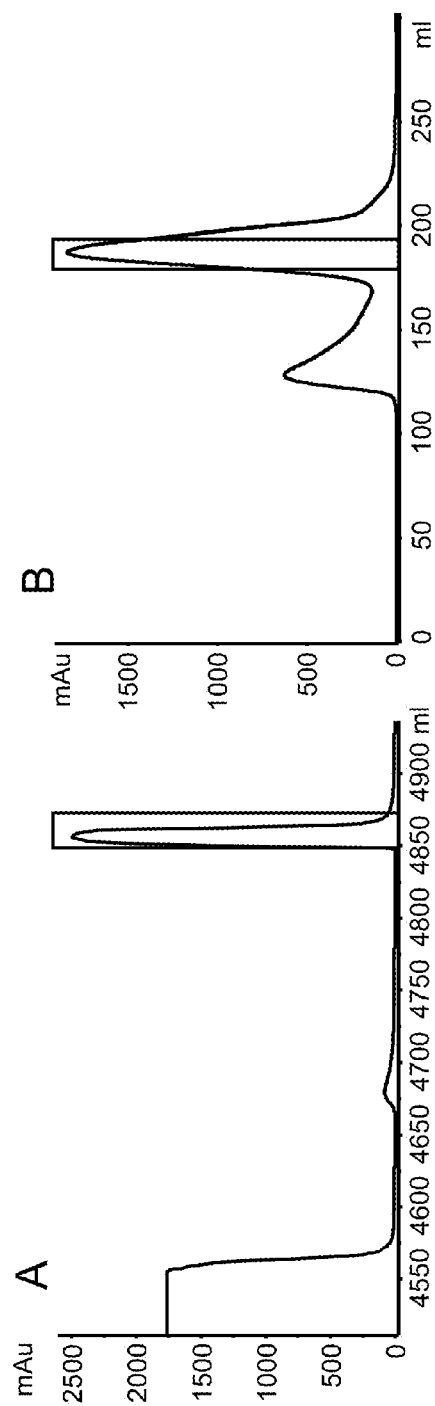


Figure 9

Figure 10

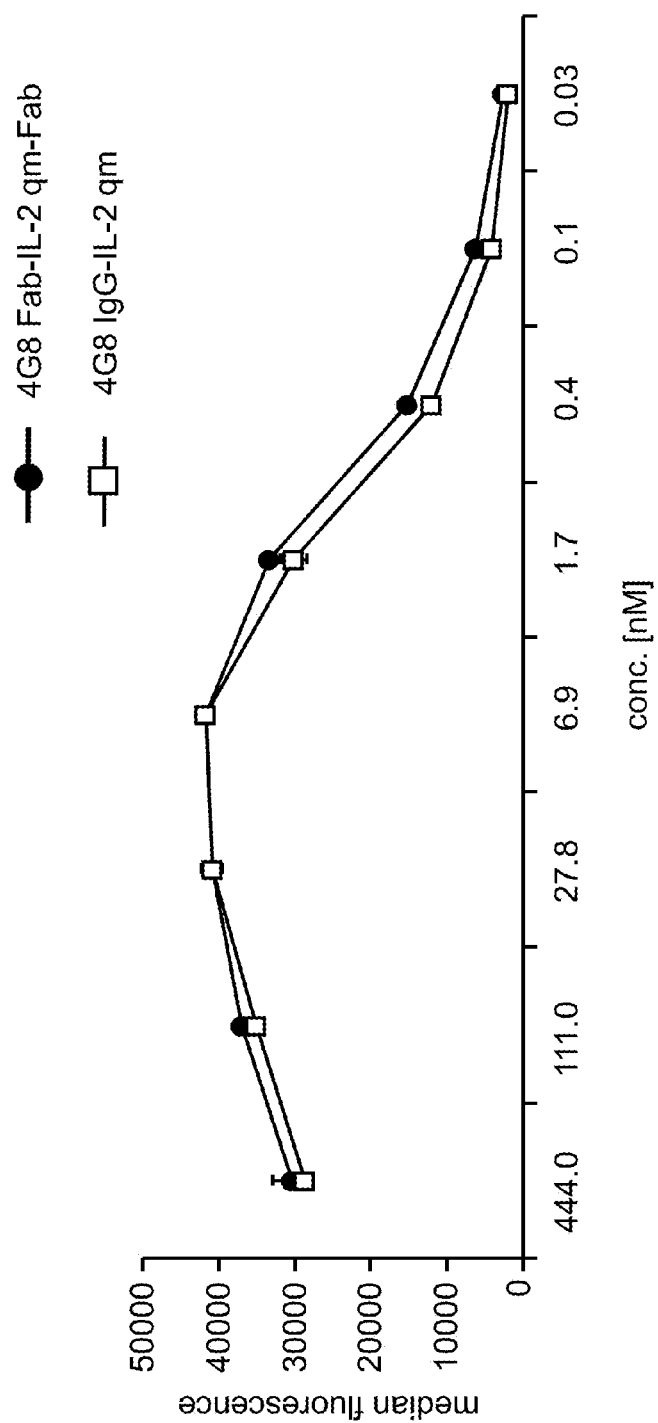


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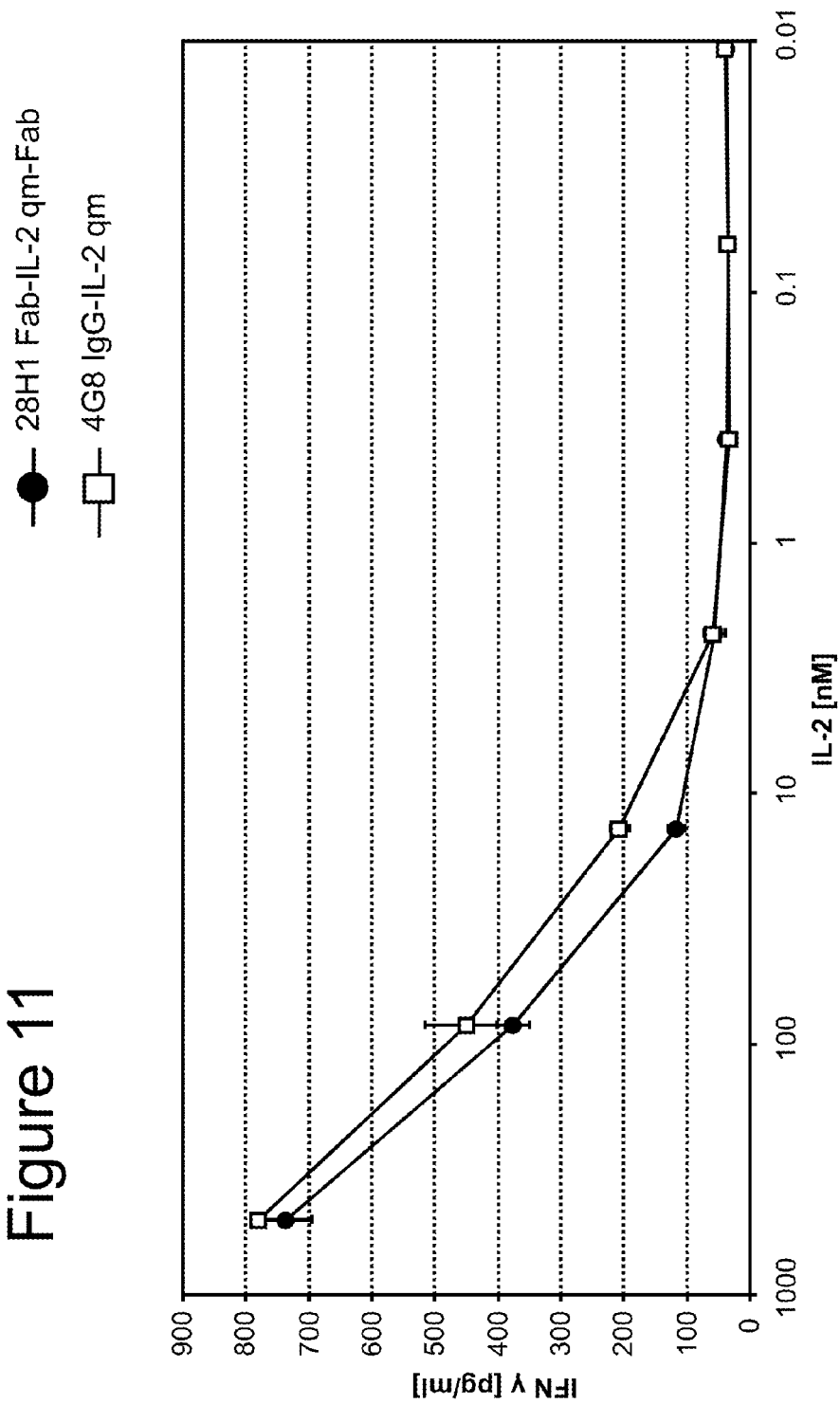


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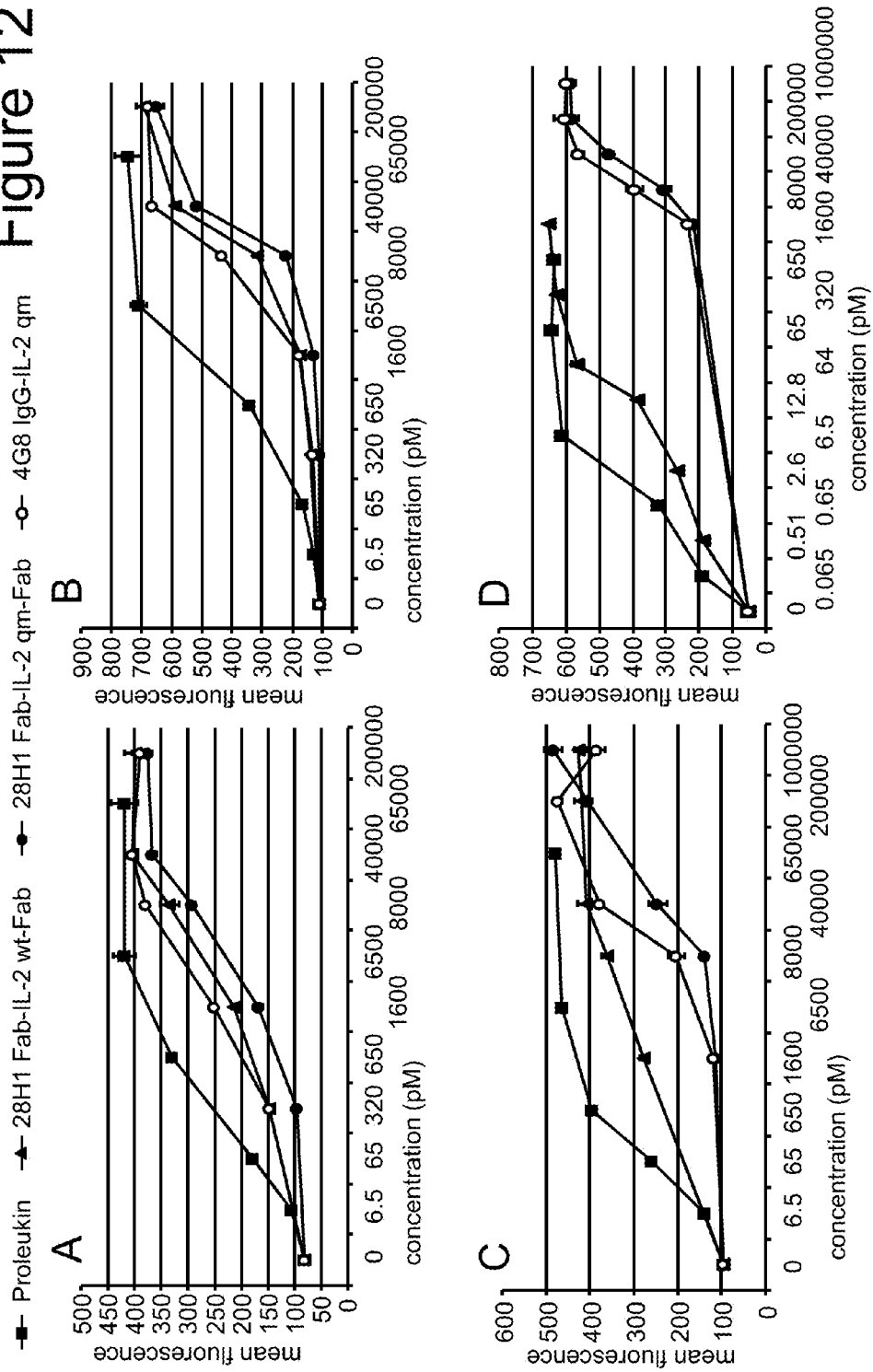
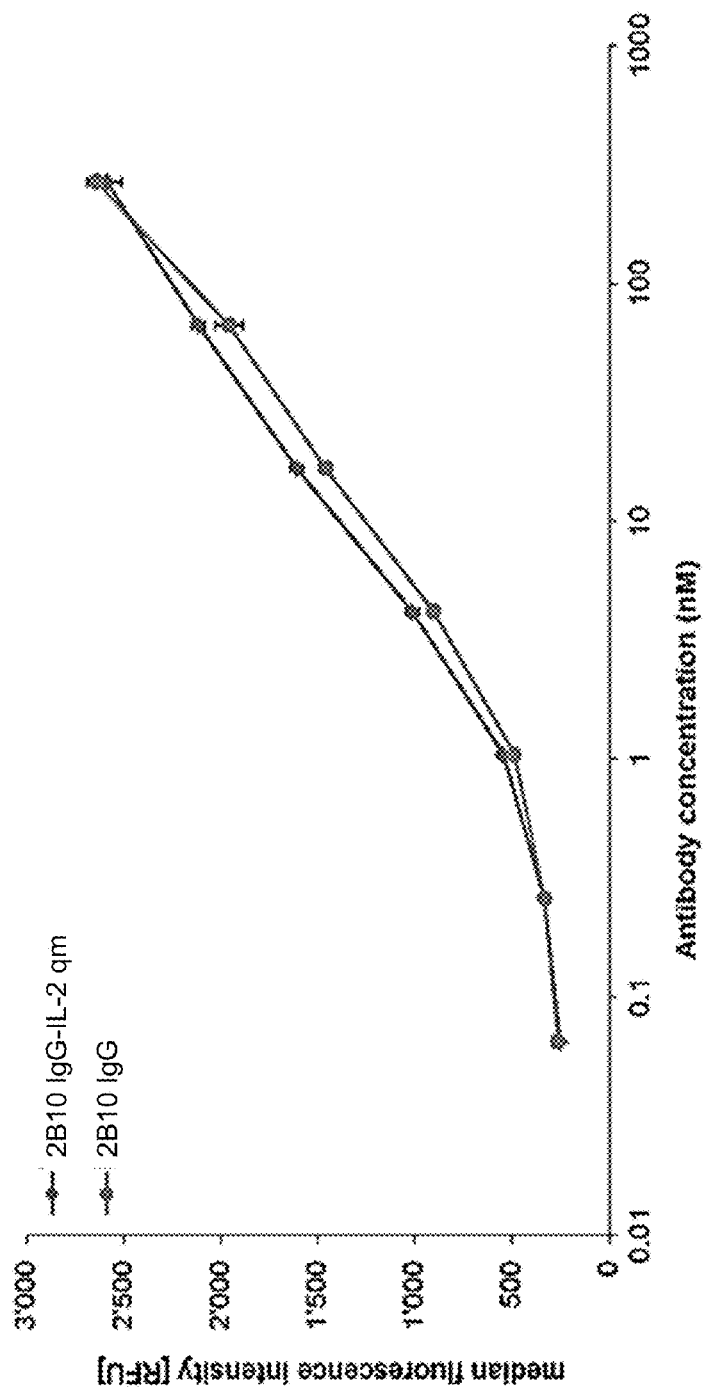


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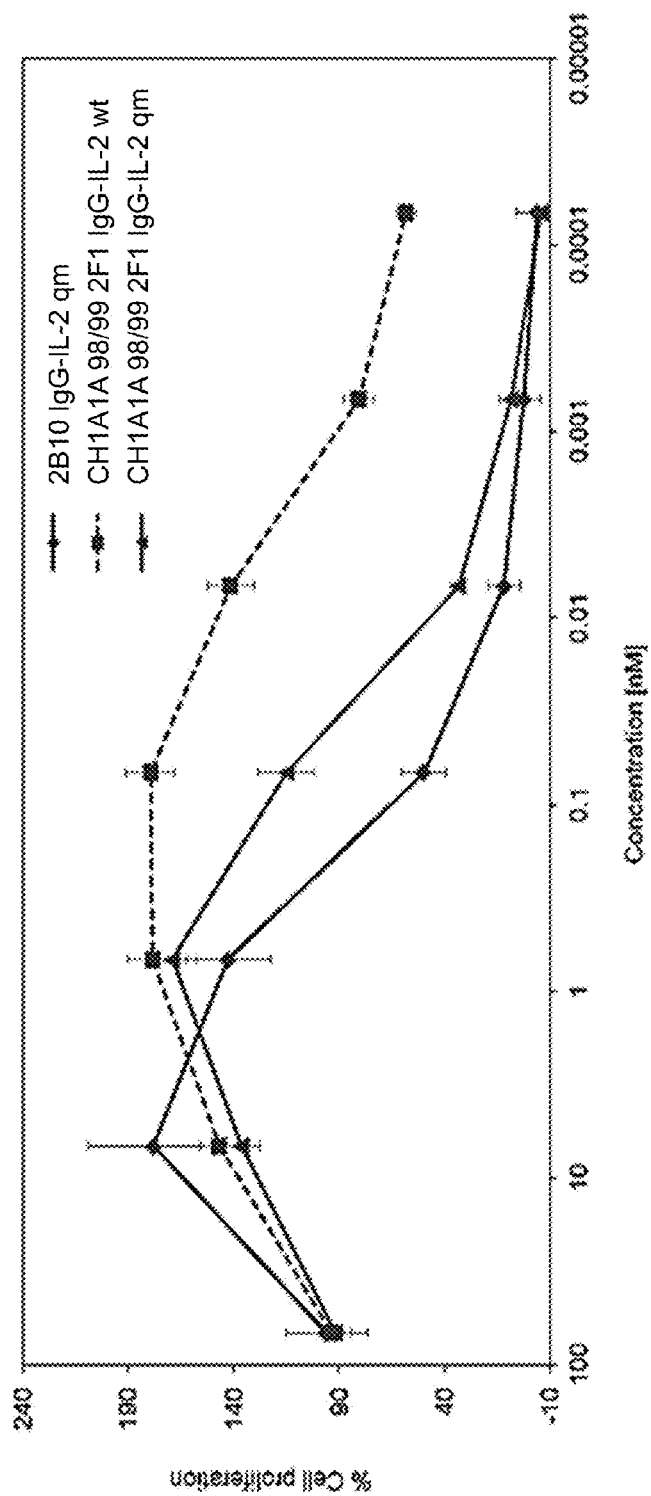


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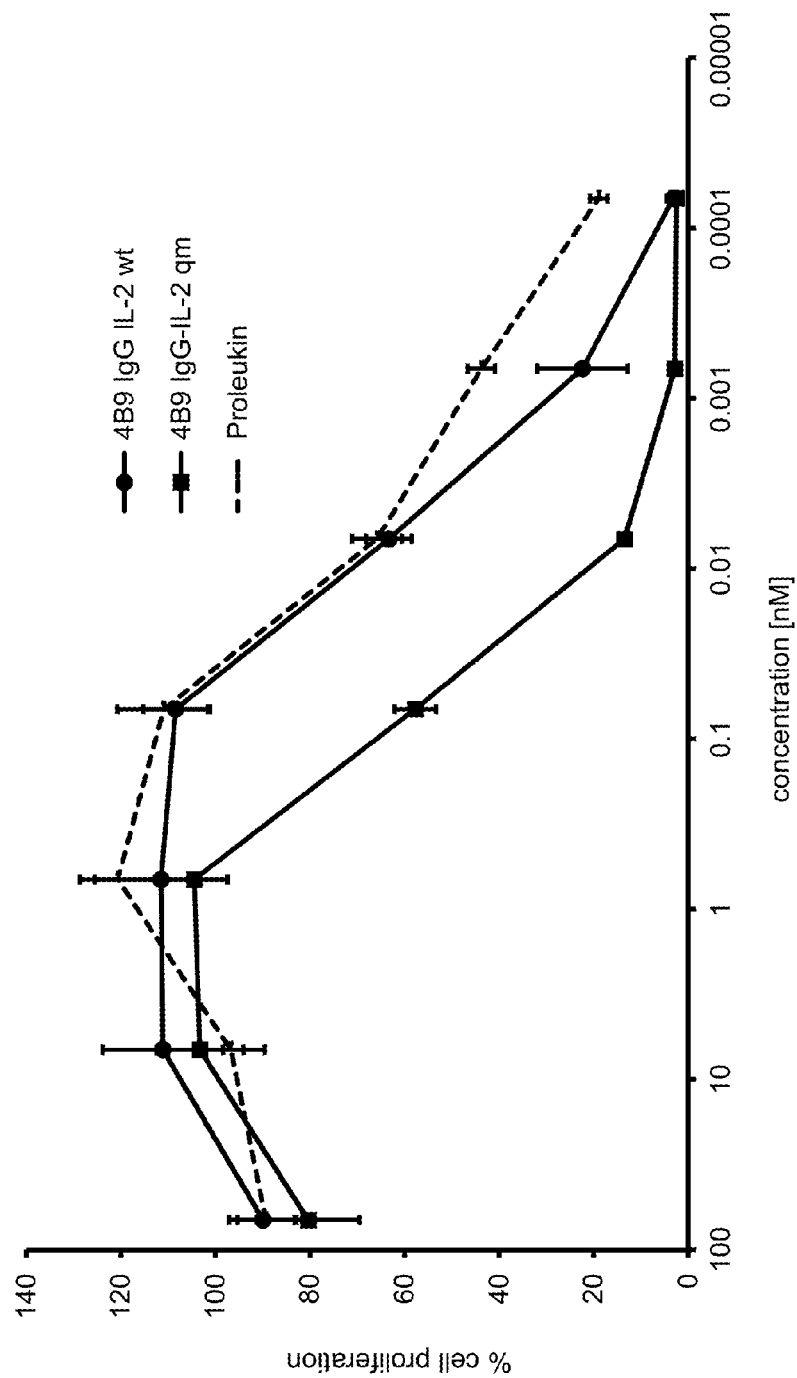


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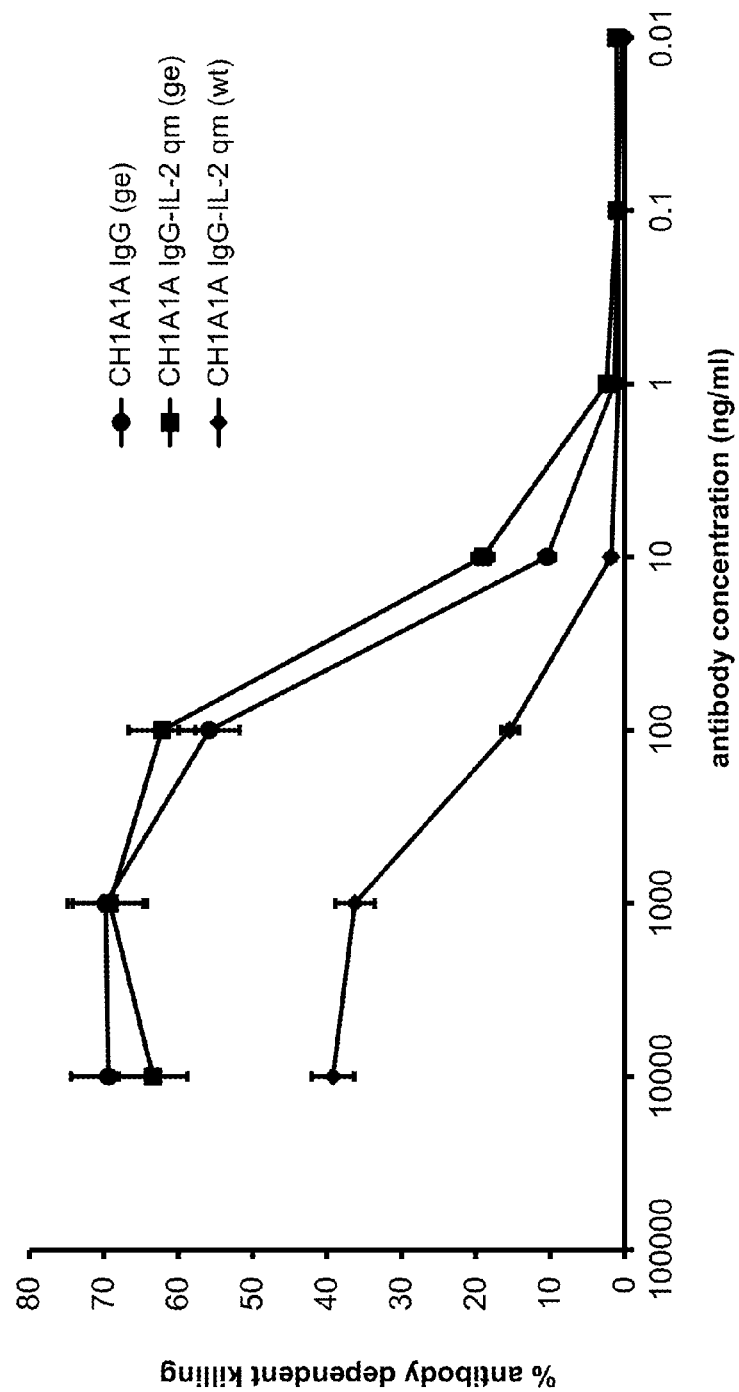


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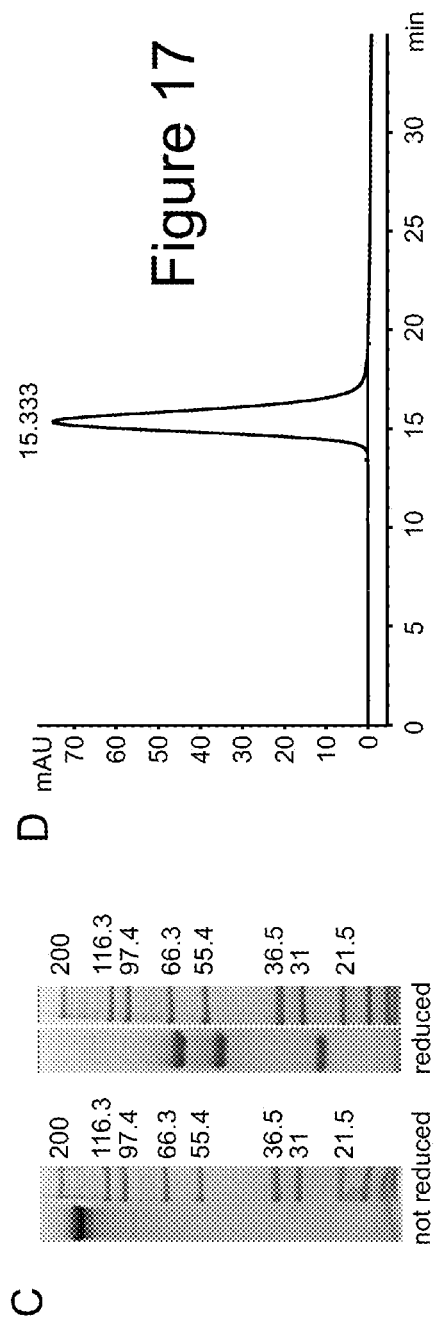
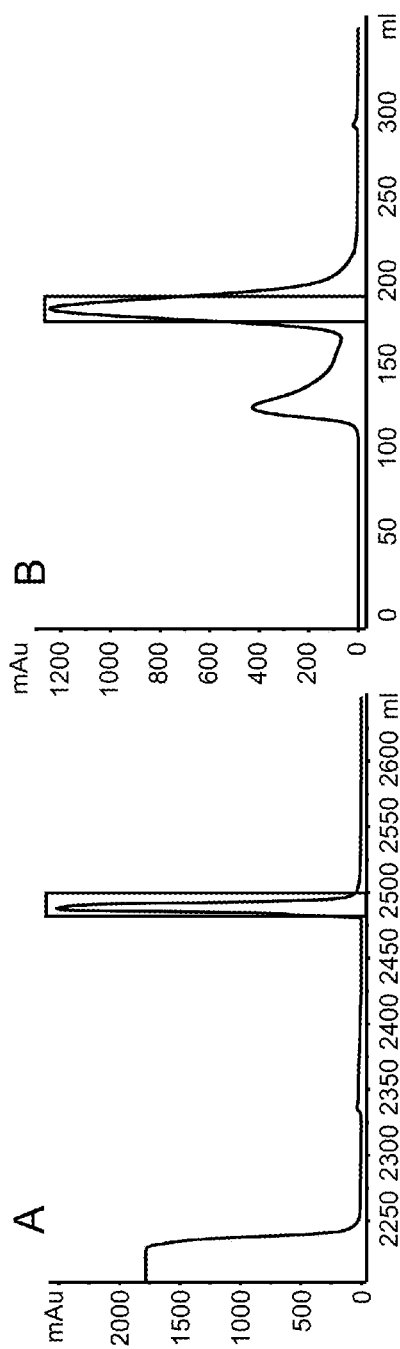


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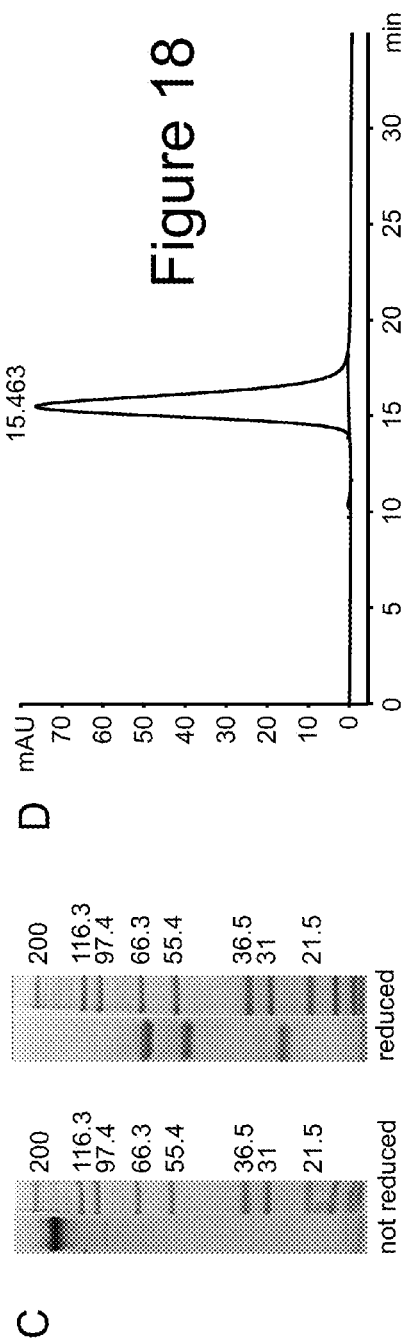
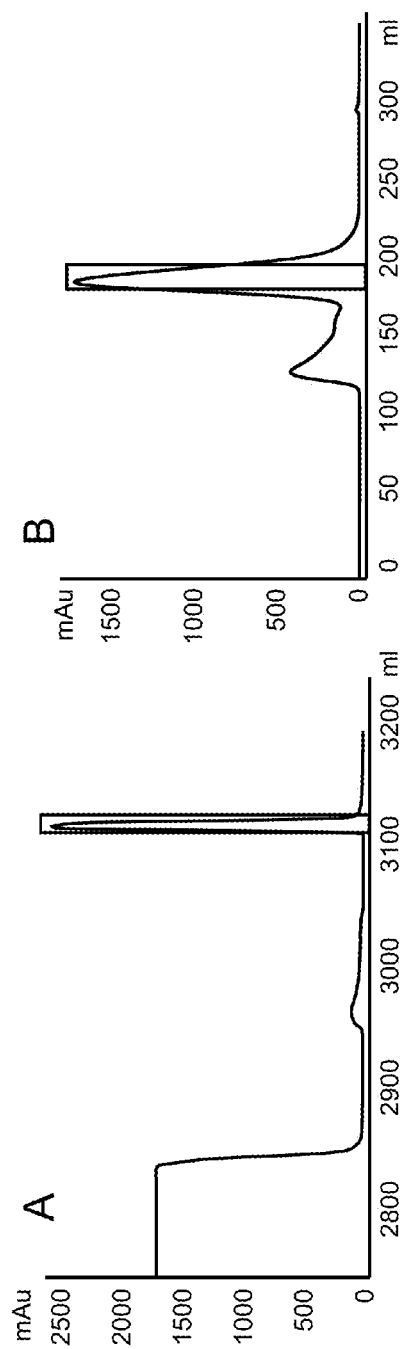
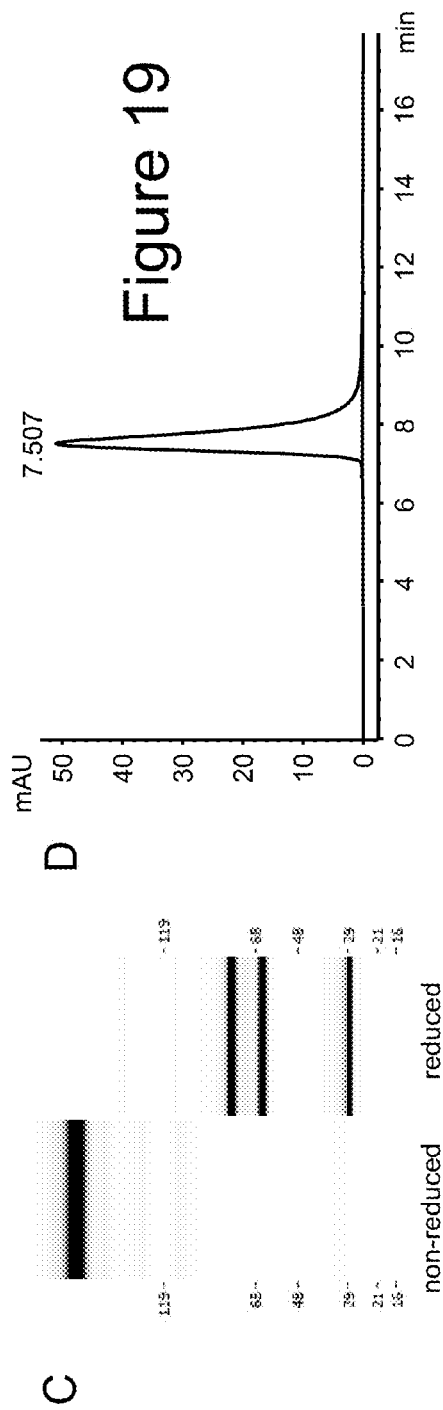
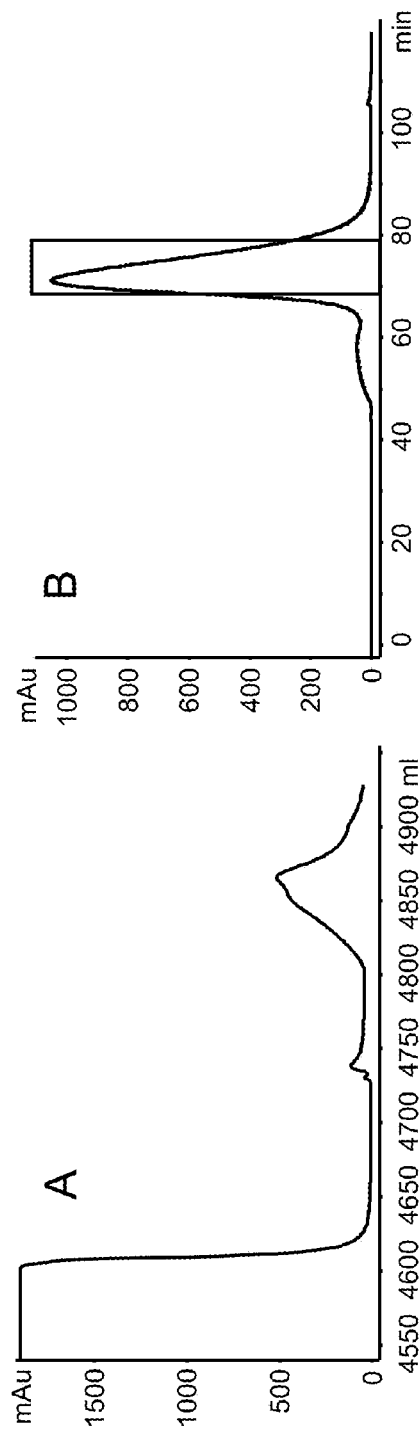


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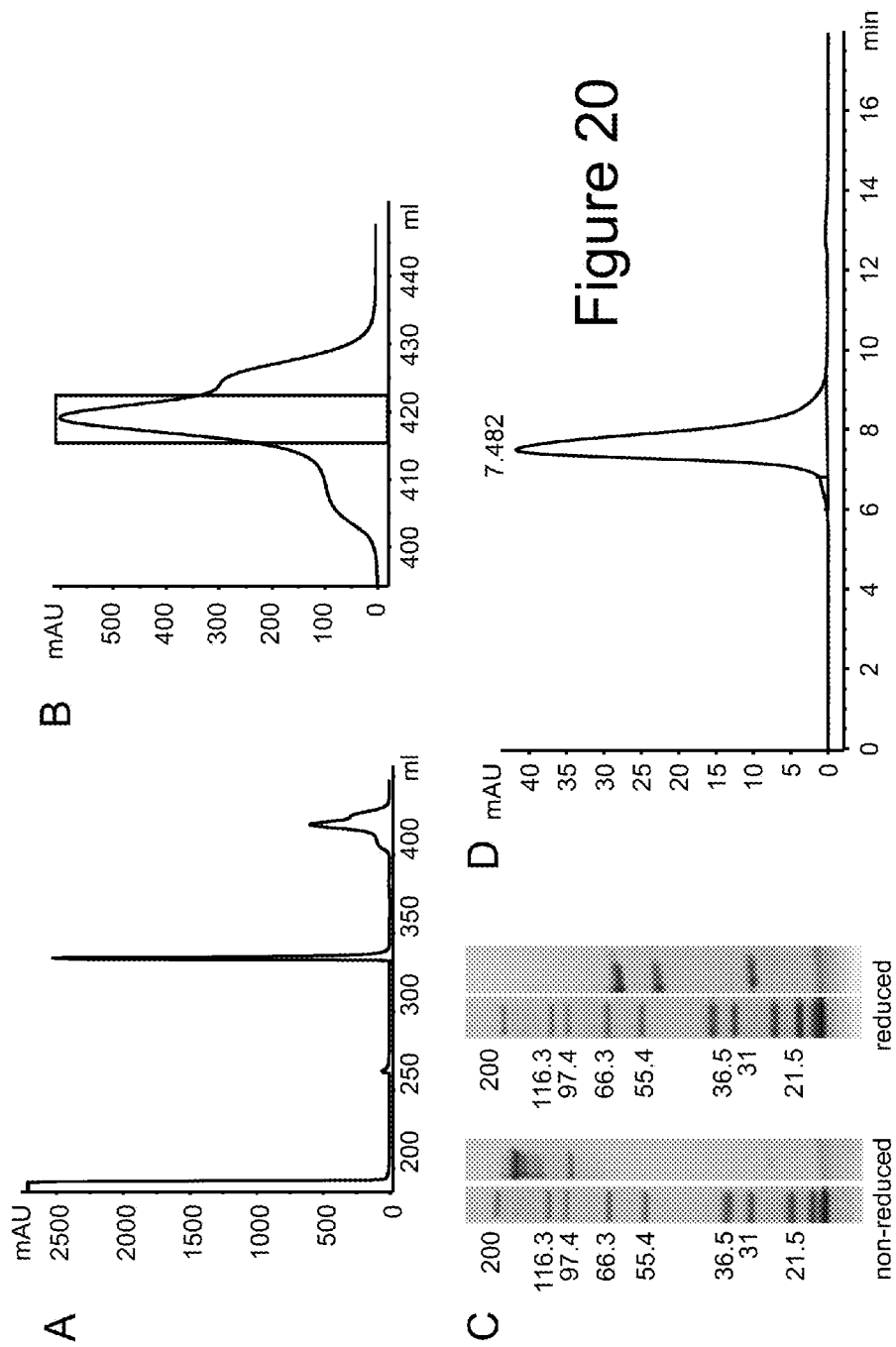


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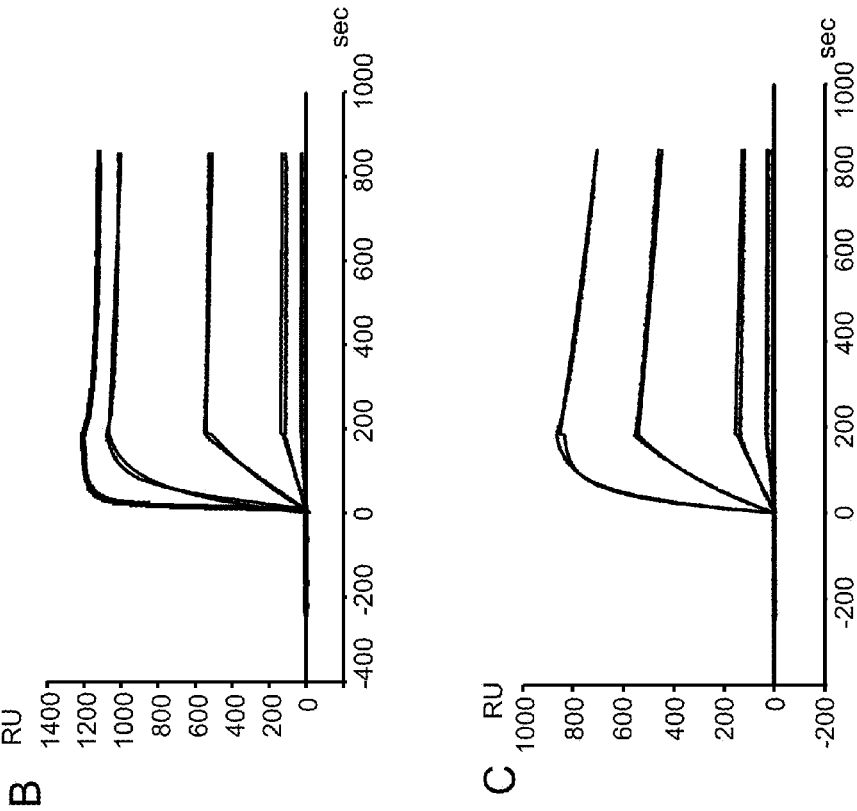
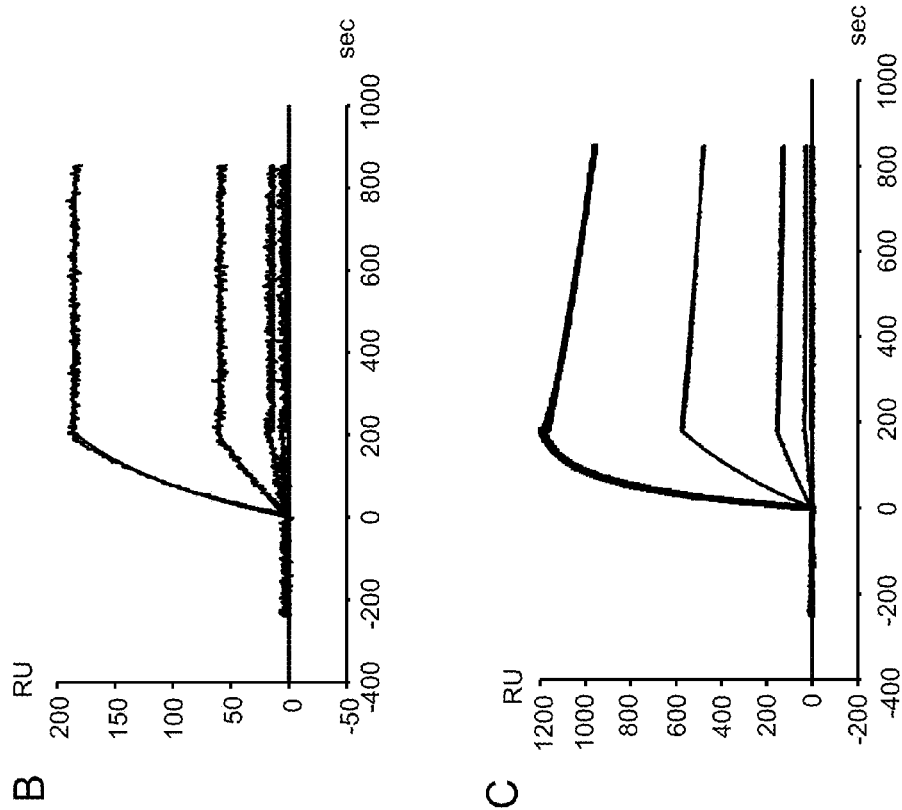
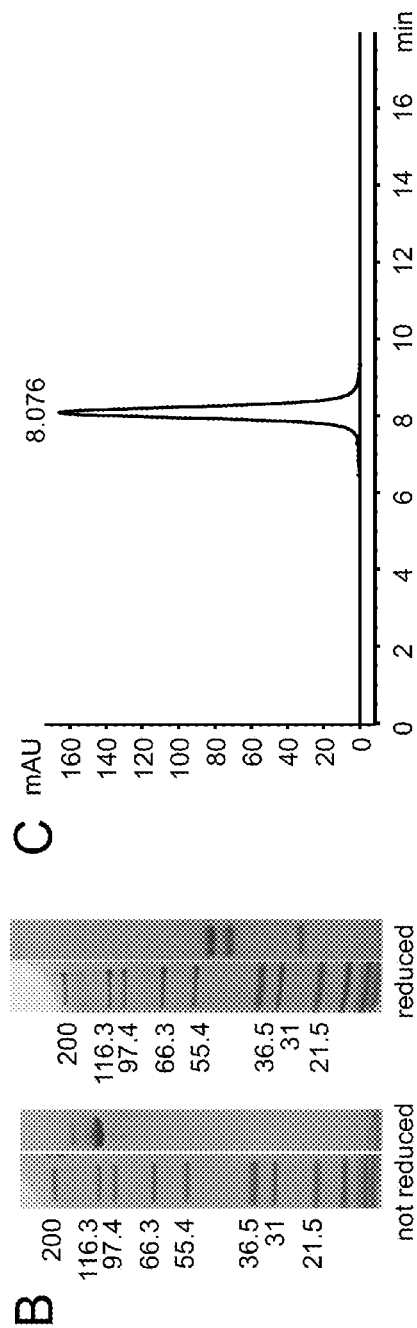
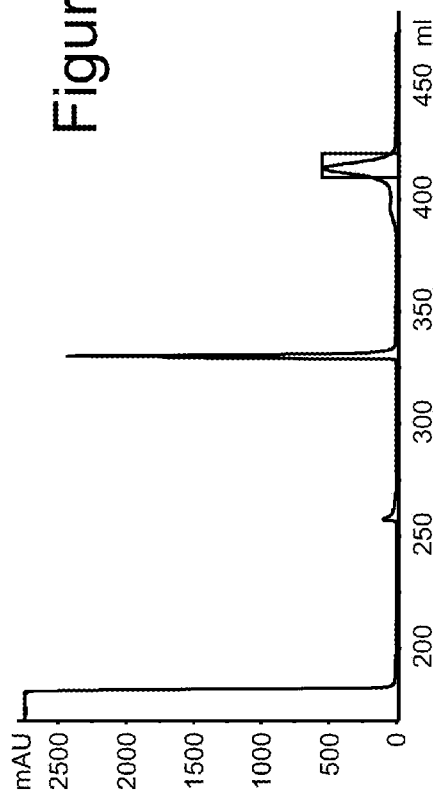


Figure 22



**A** **Figure 23**



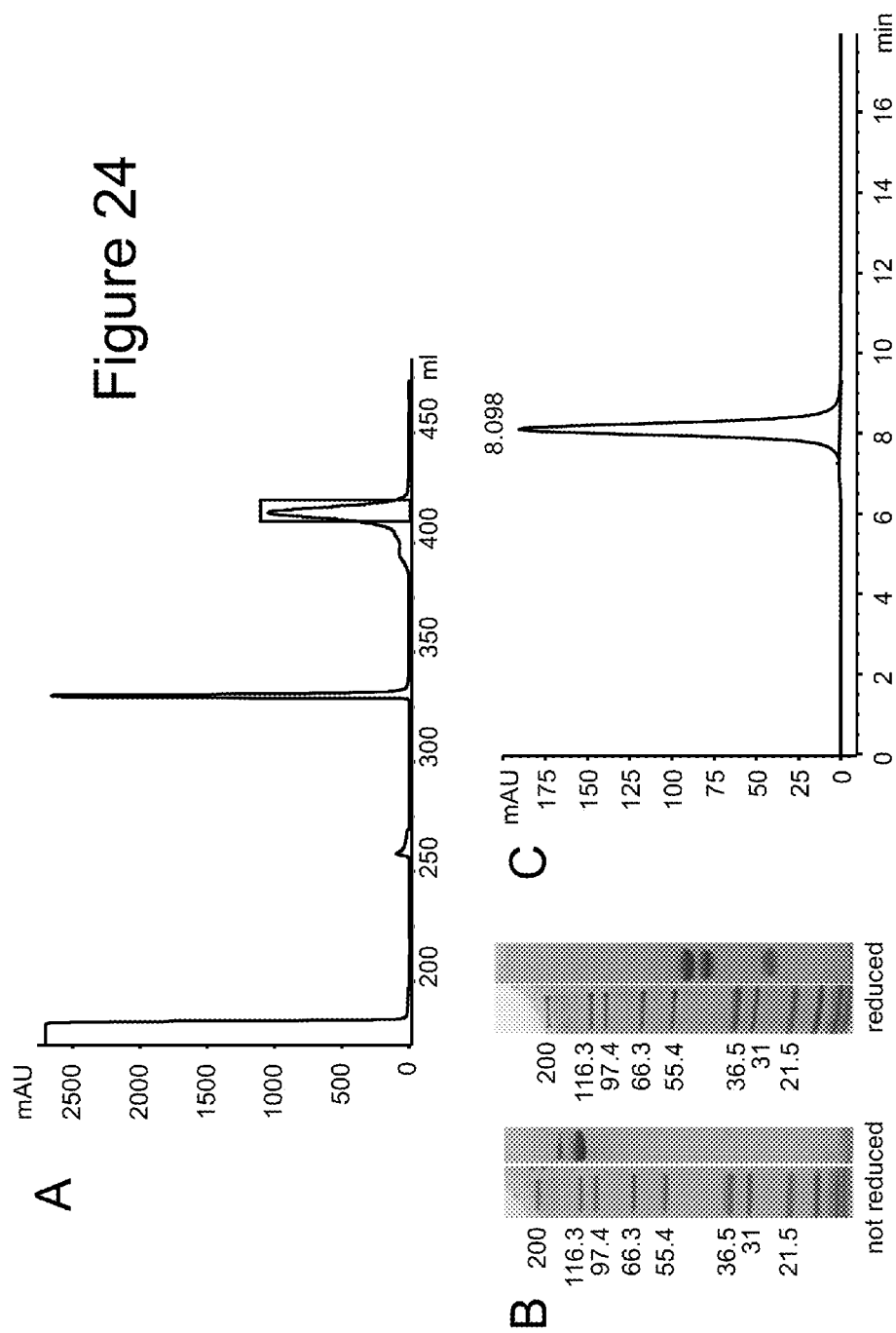
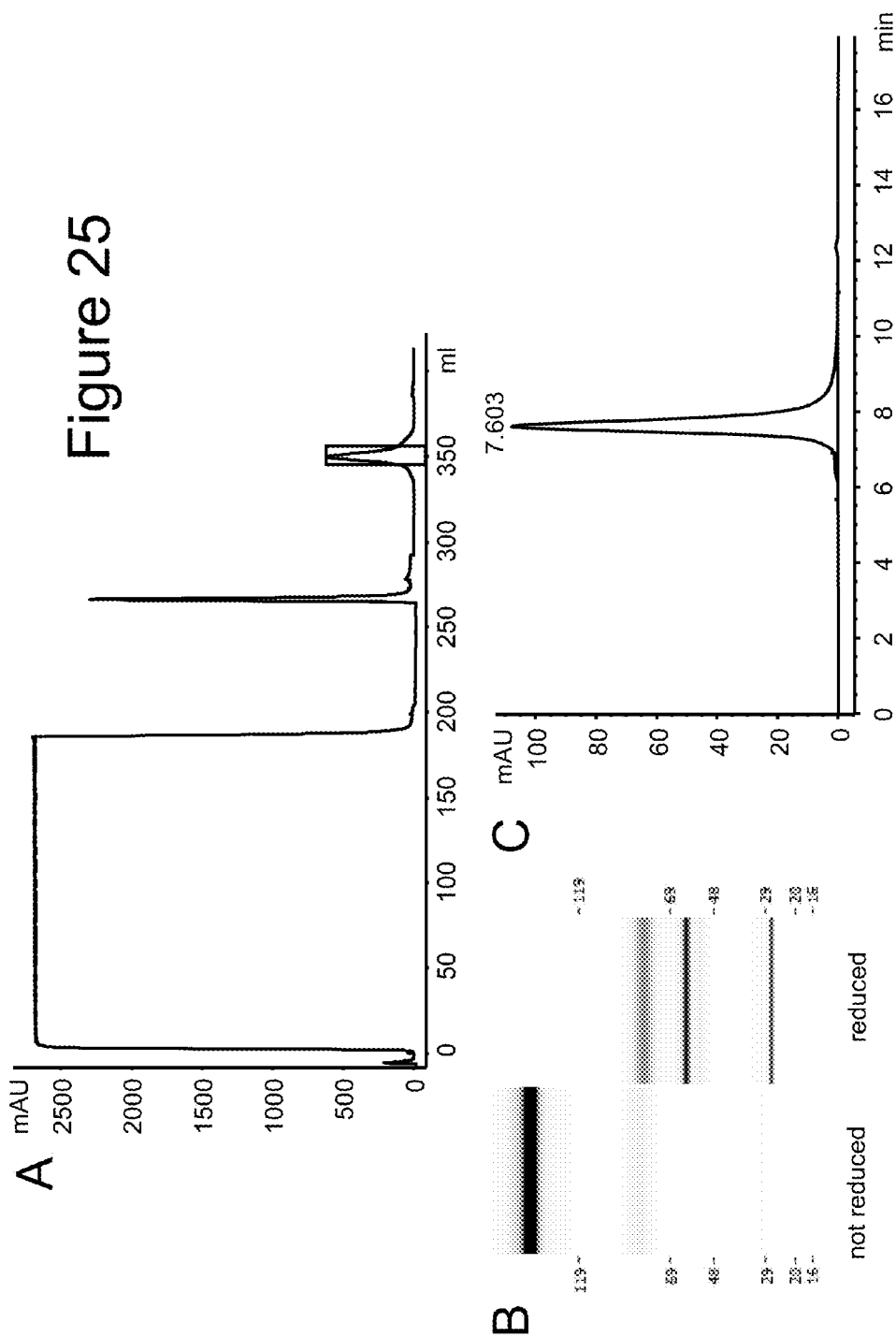
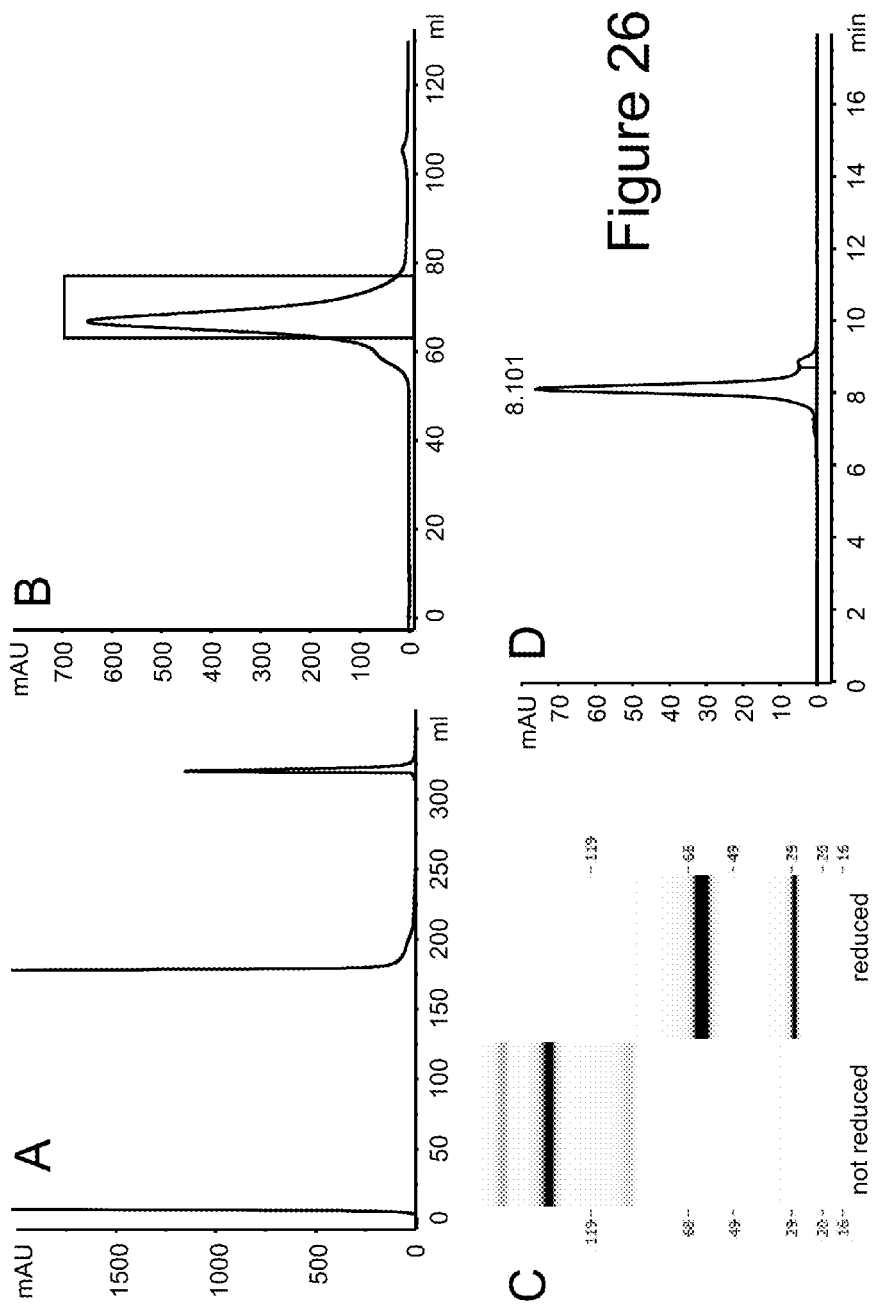


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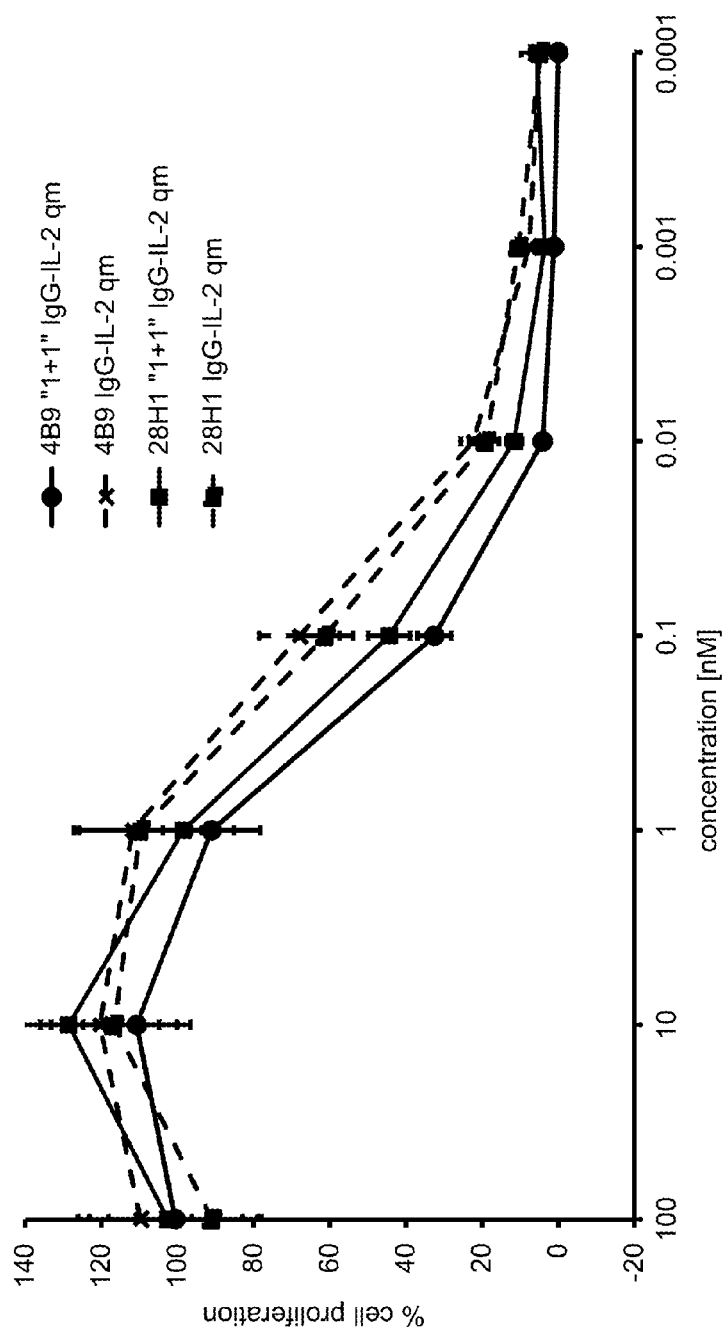


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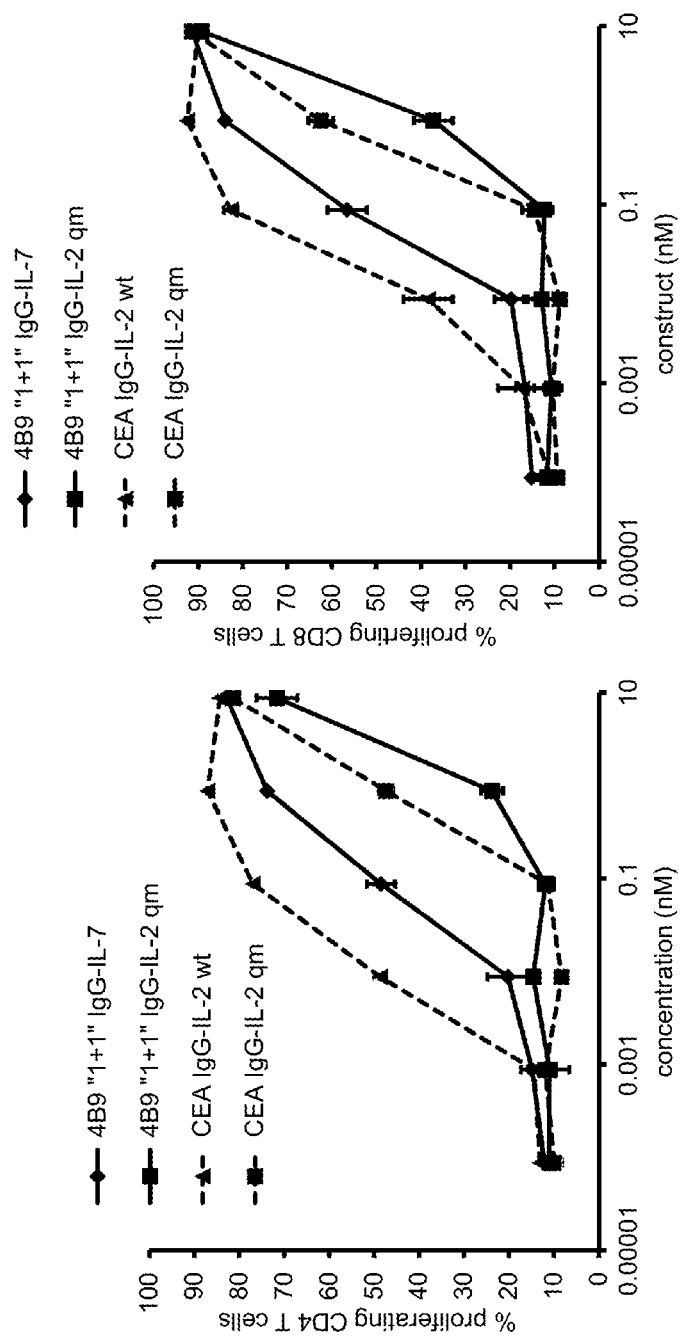


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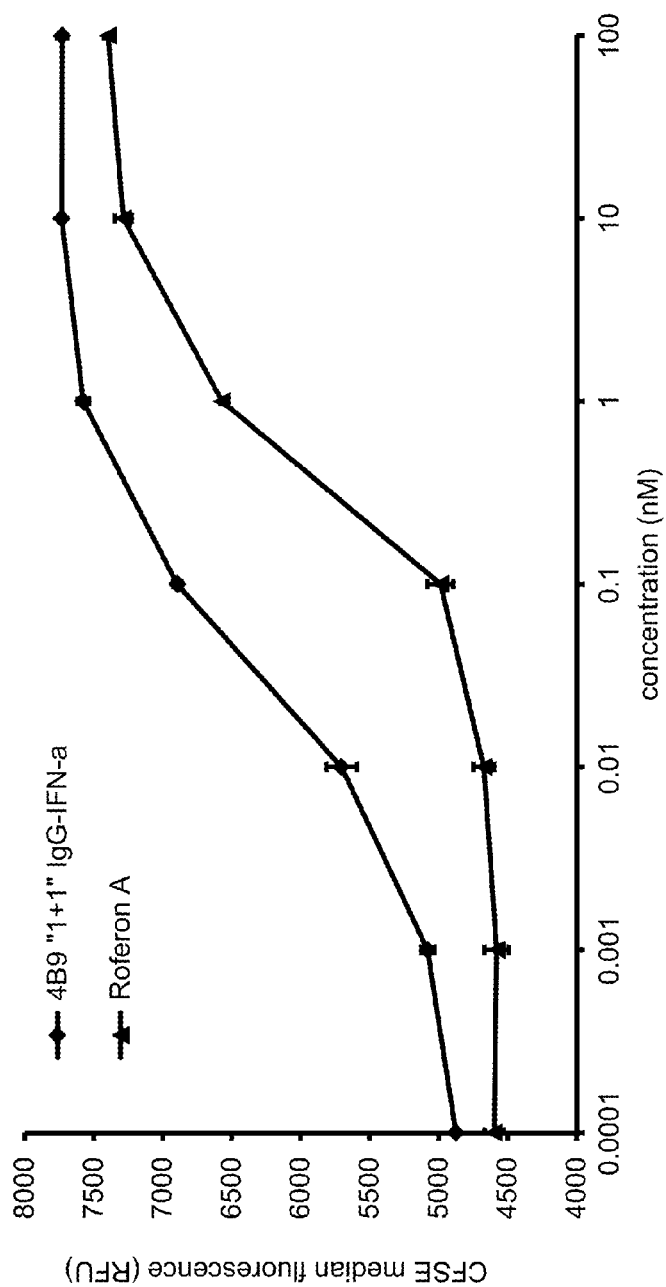


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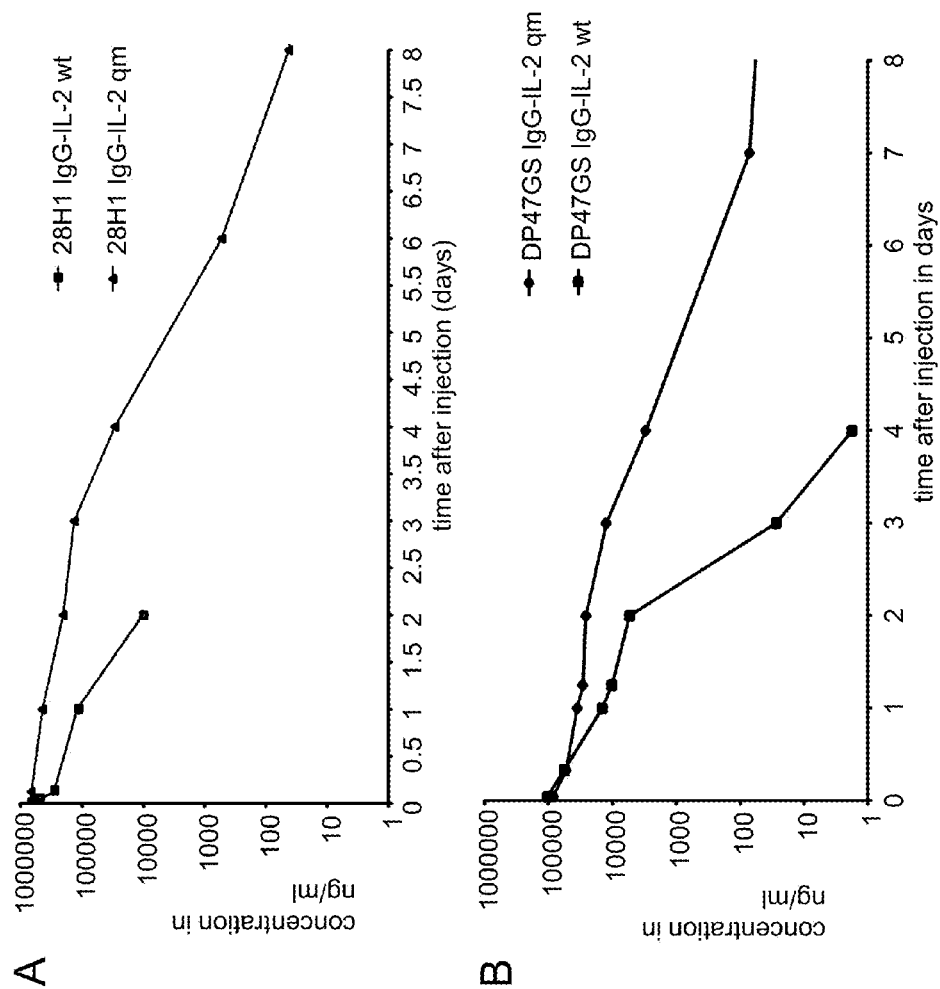


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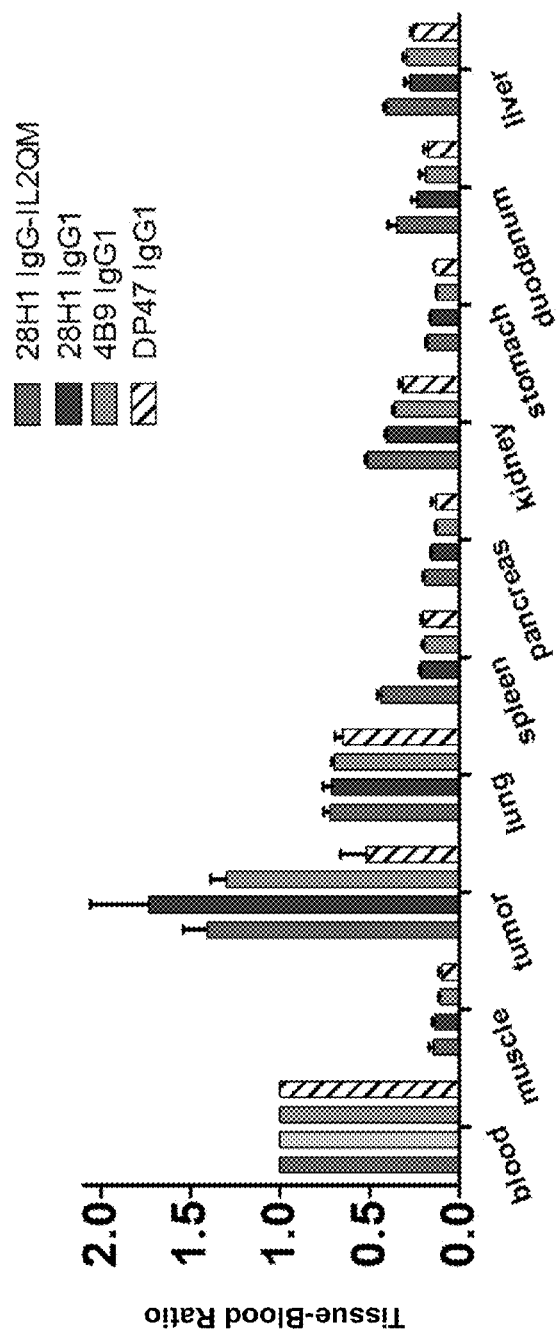


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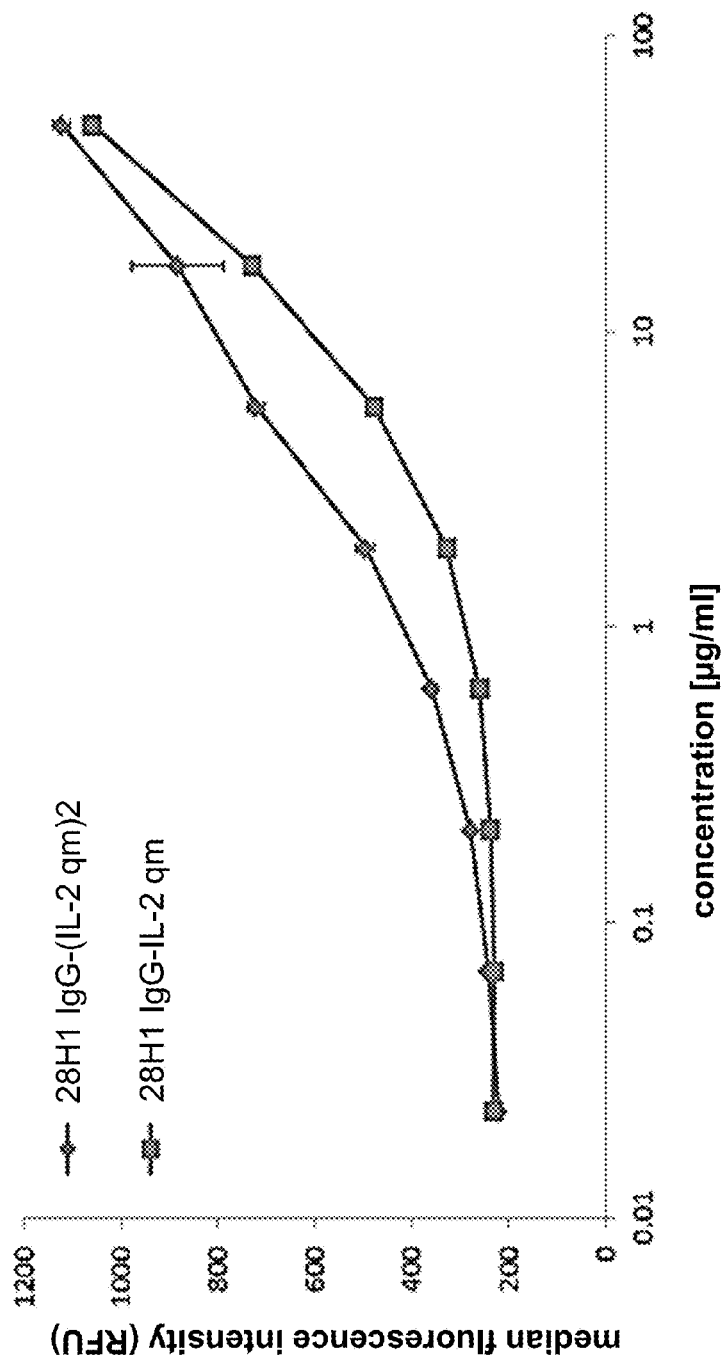


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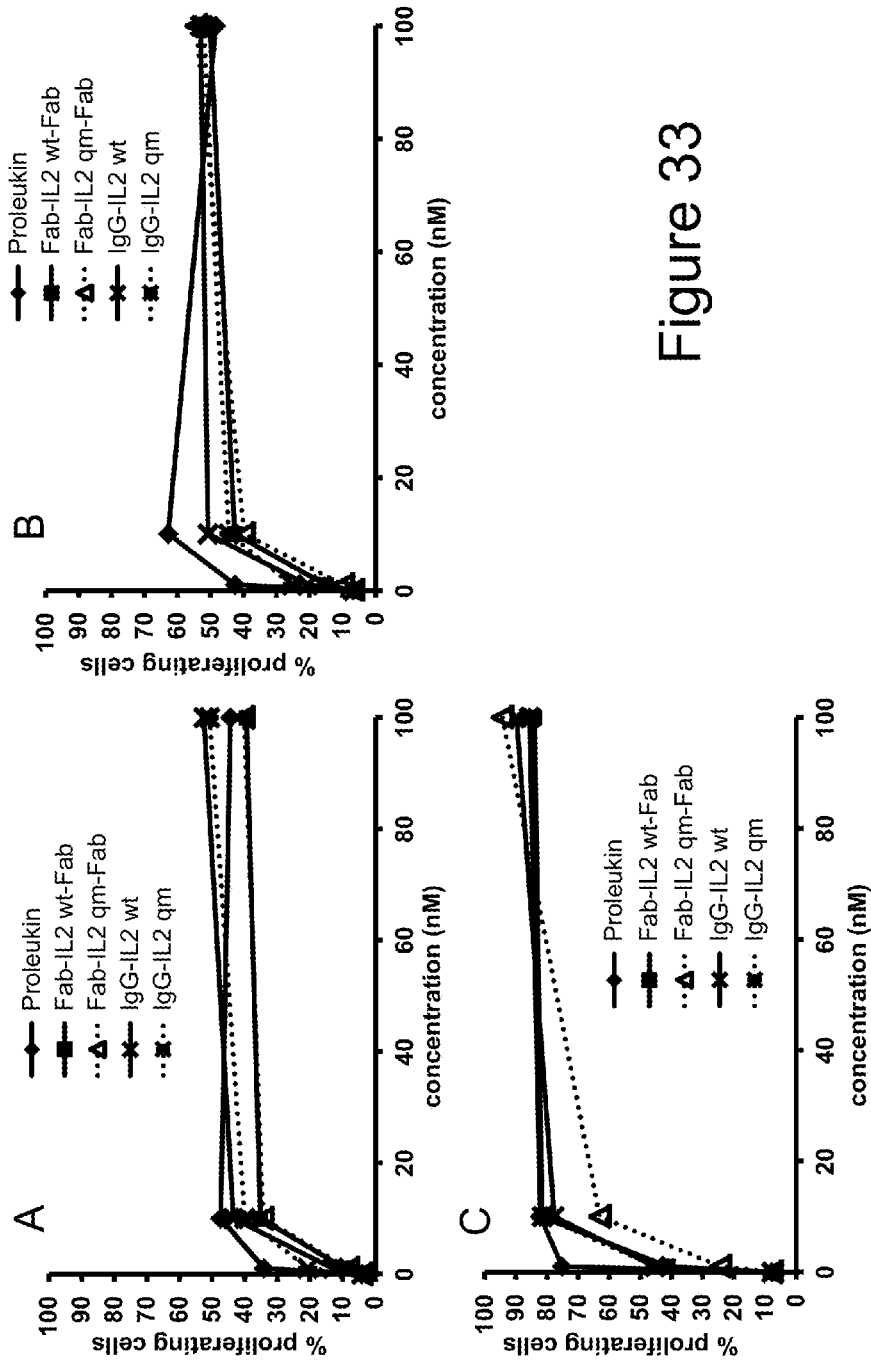


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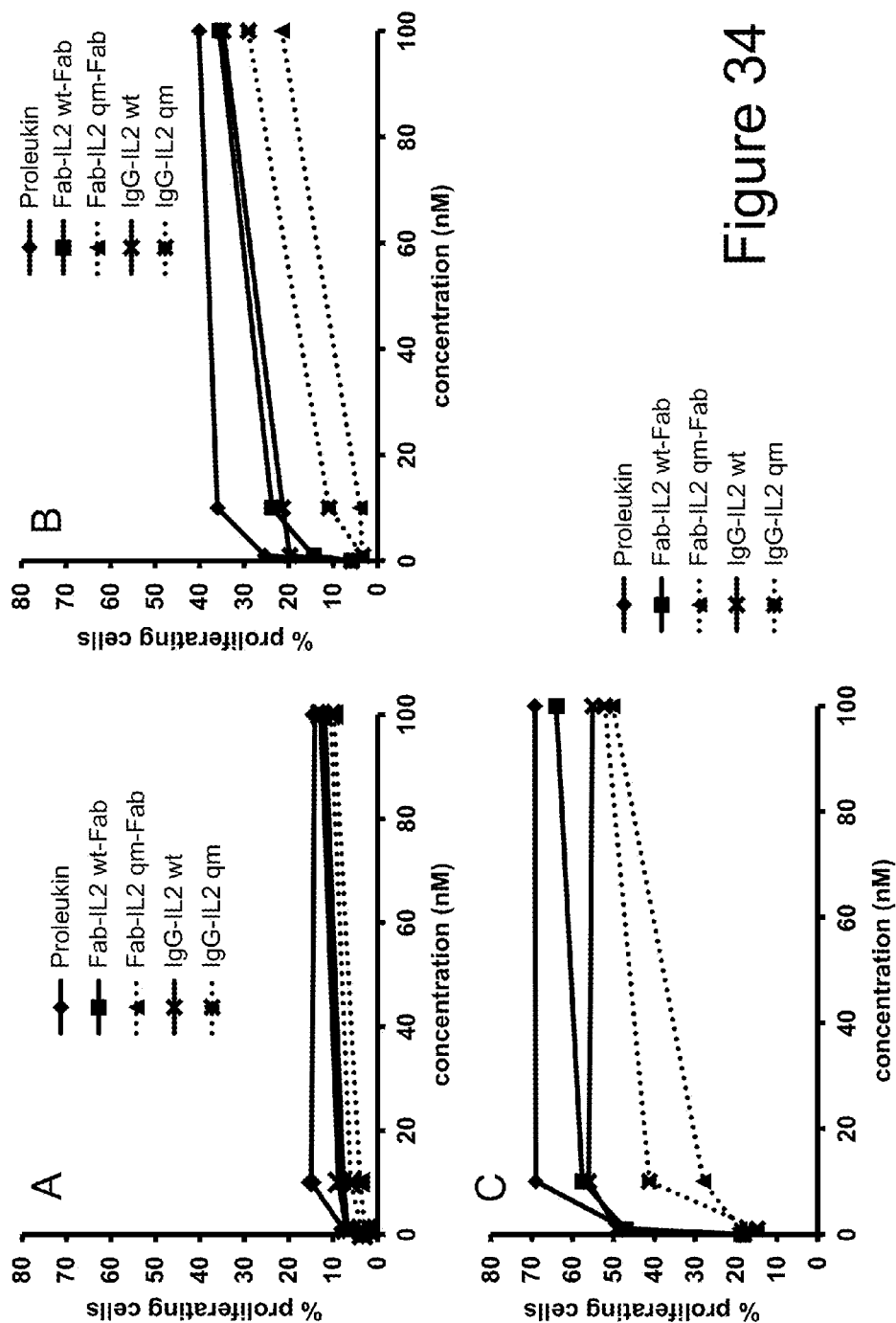


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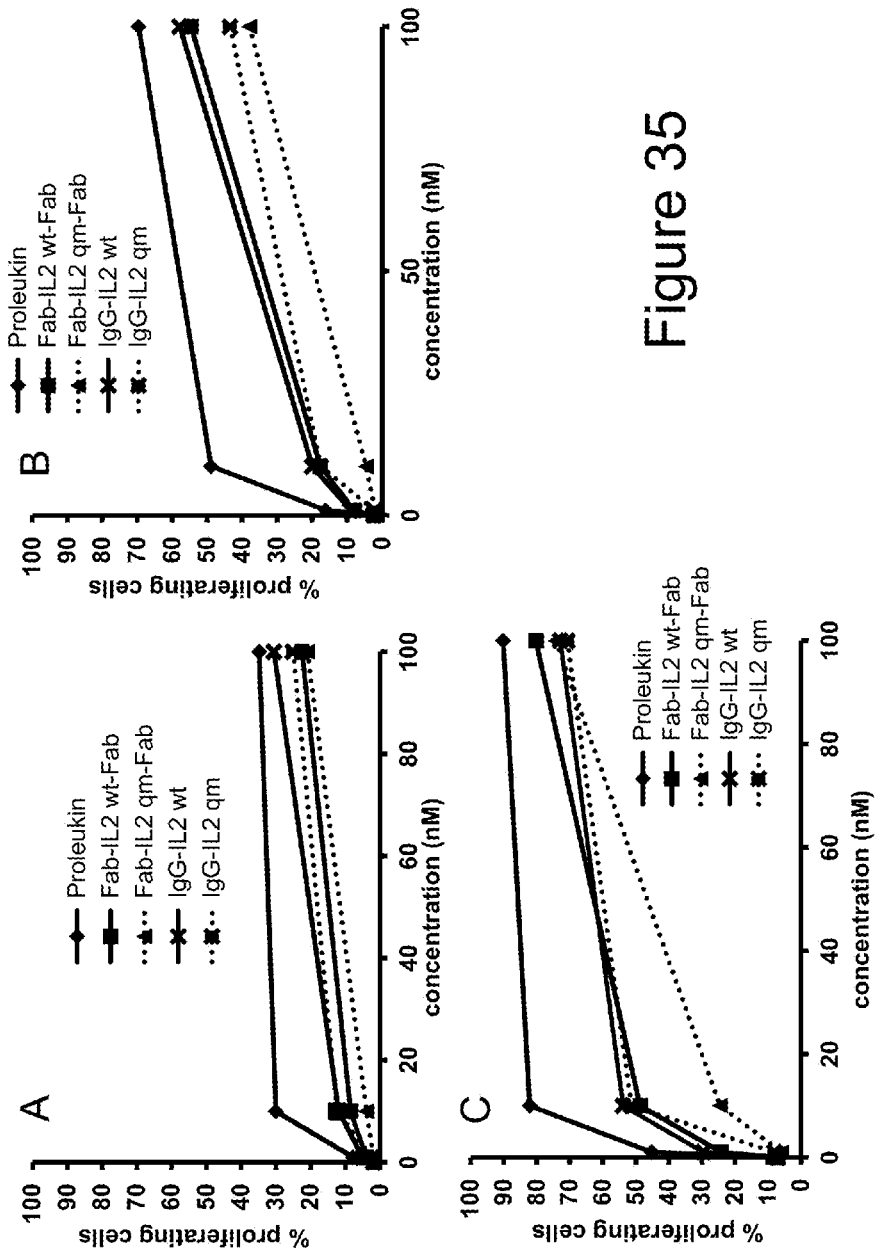


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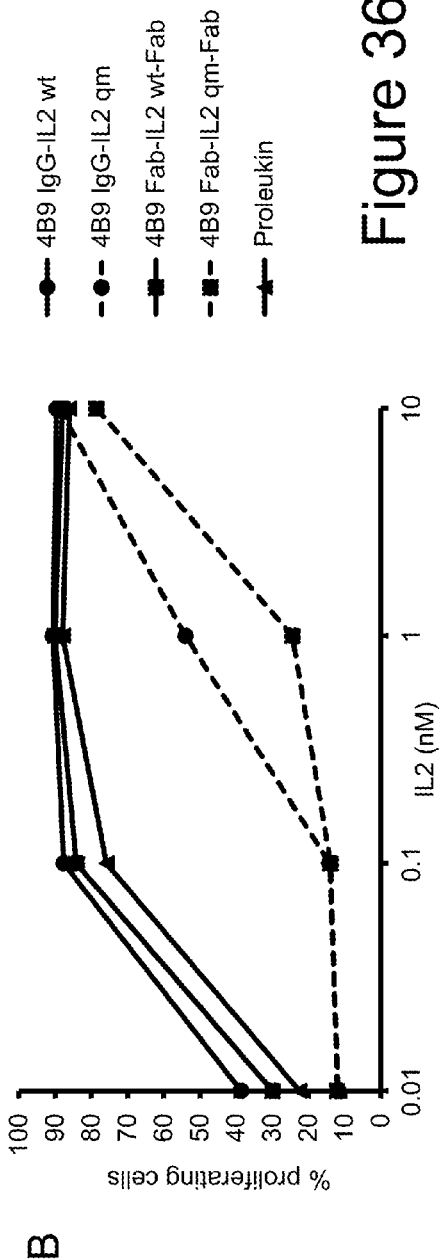
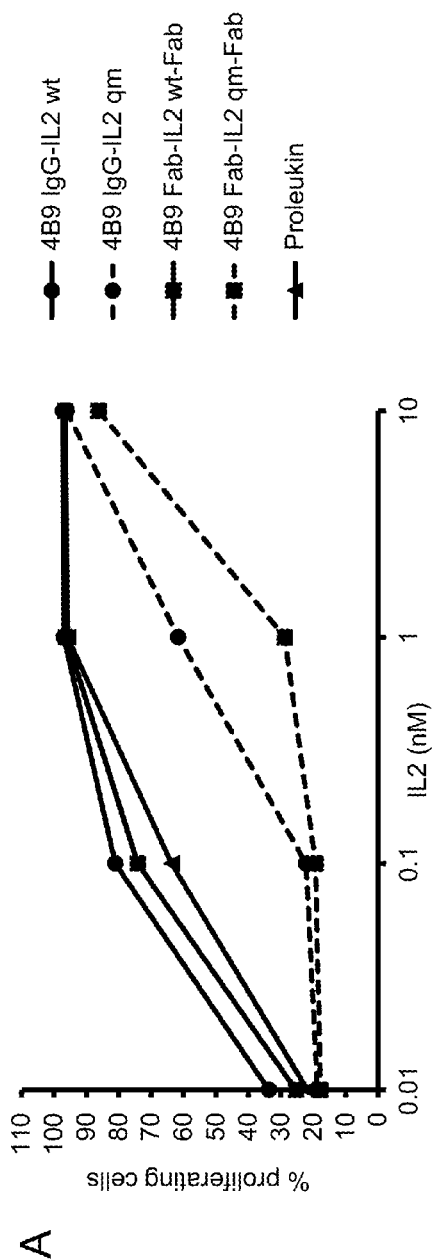


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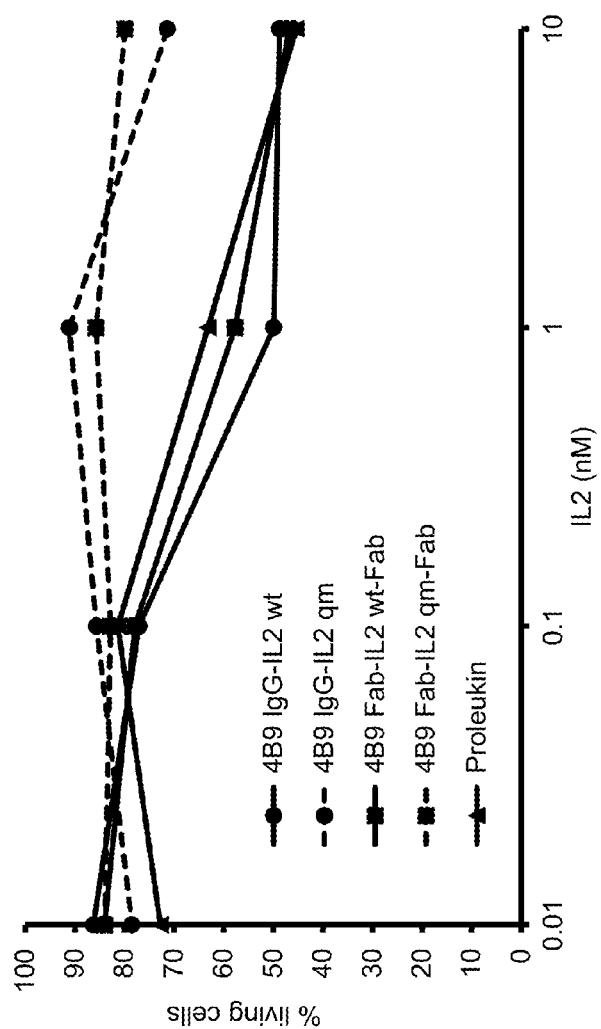


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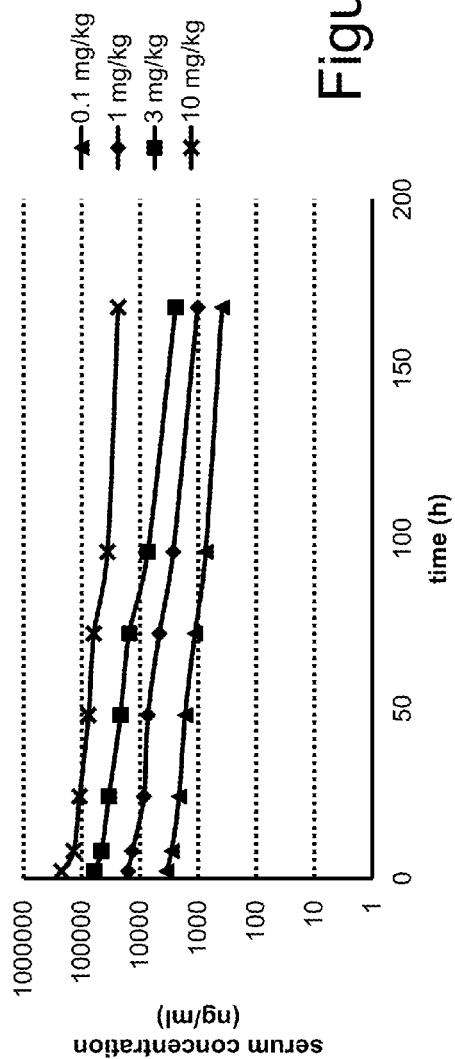
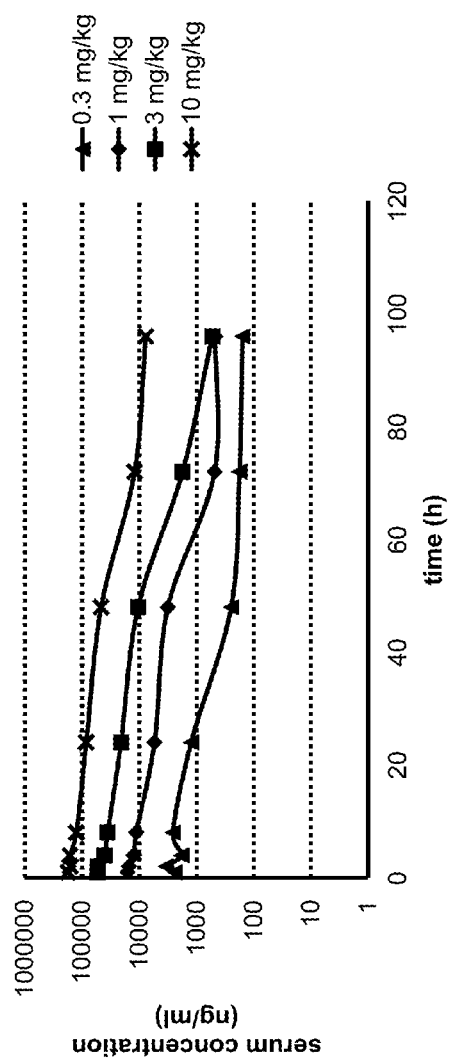


Figure 38

Figure 39

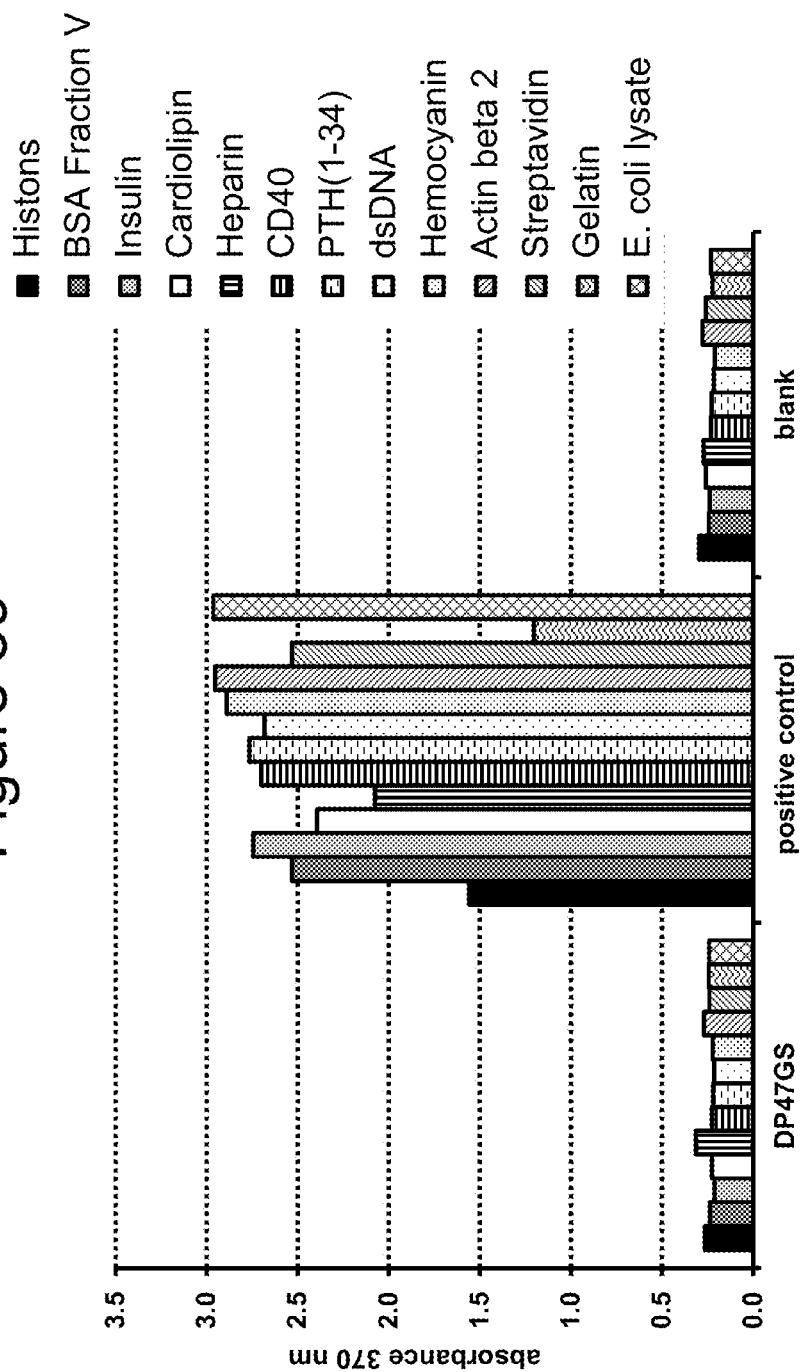


Figure 40A

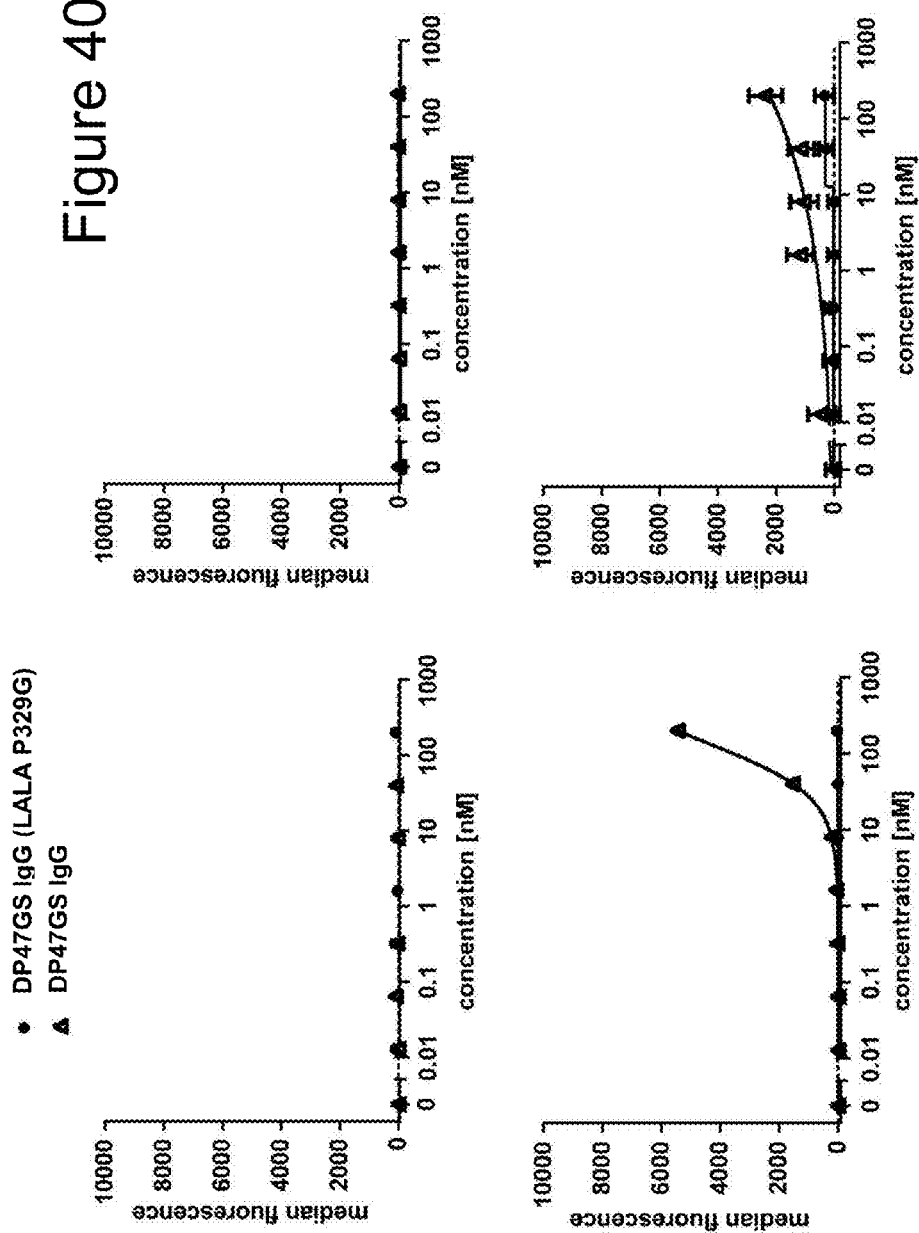


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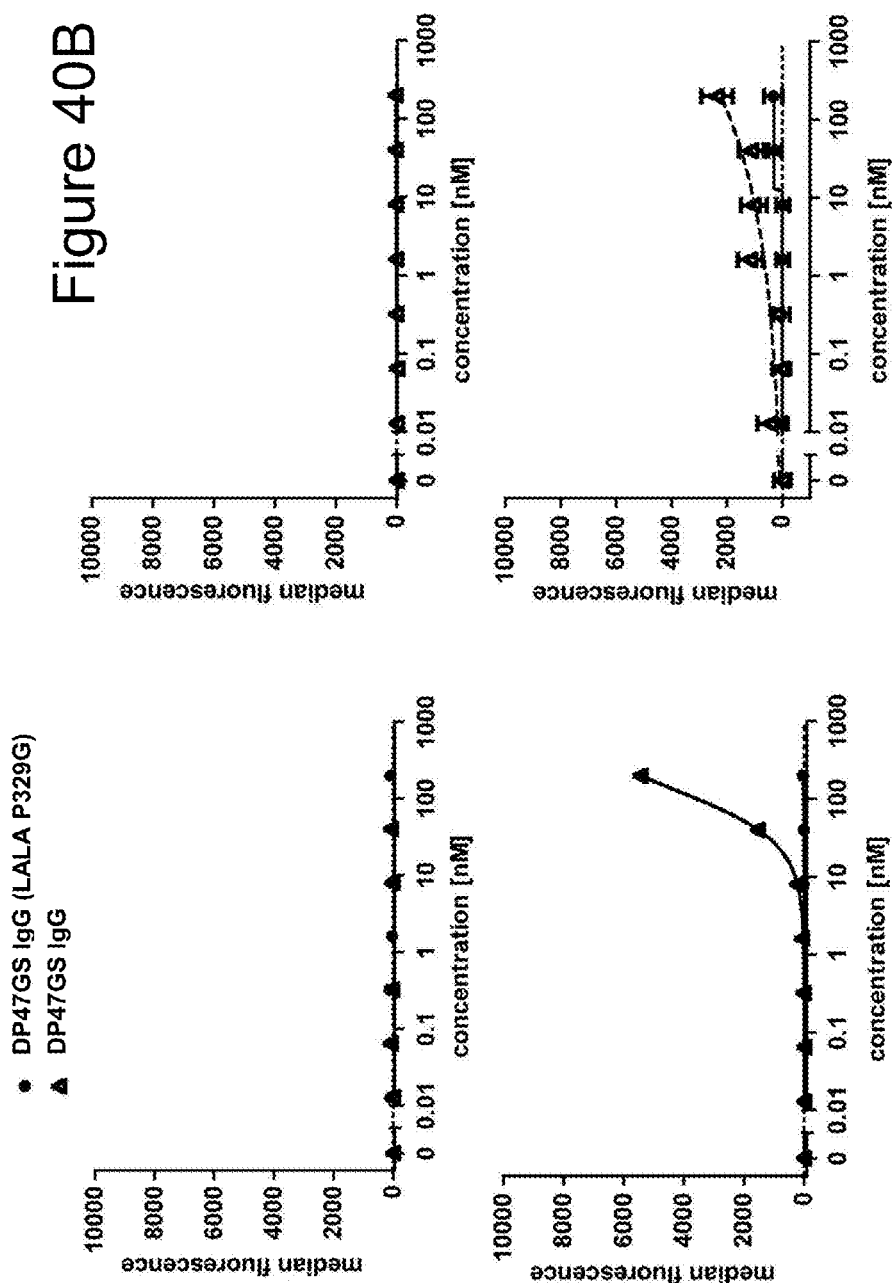
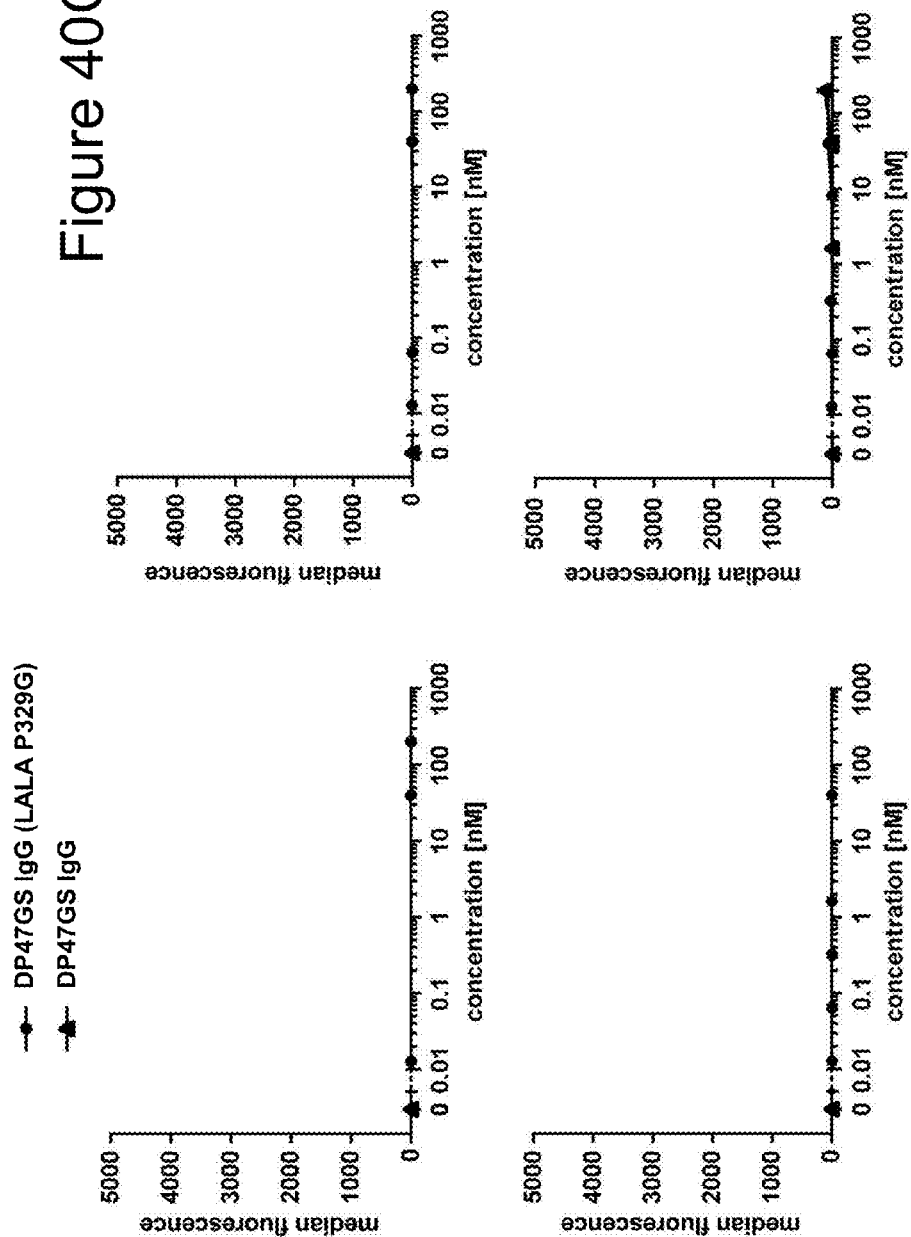


Figure 40C



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## IMMUNOCONJUGATES

## FIELD OF THE INVENTION

The present invention generally relates to antigen-specific immunoconjugates for selectively delivering effector moieties that influence cellular activity. In addition, the present invention relates to polynucleotides encoding such immunoconjugates, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the immunoconjugates of the invention, and to methods of using these immunoconjugates in the treatment of disease.

## BACKGROUND

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. A multitude of signal transduction pathways in the cell are linked to the cell's survival and/or death. Accordingly, the direct delivery of a pathway factor involved in cell survival or death can be used to contribute to the cell's maintenance or destruction. Similarly, specific factors may be delivered that stimulate immune effector cells in a tumor microenvironment, such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs), to attack and destroy tumor cells.

Cytokines are cell signaling molecules that participate in regulation of the immune system. When used in cancer therapy, cytokines can act as immunomodulatory agents that have anti-tumor effects and which can increase the immunogenicity of some types of tumors. However, rapid blood clearance and lack of tumor specificity require systemic administration of high doses of the cytokine in order to achieve a concentration of the cytokine at the tumor site sufficient to activate an immune response or have an anti-tumor effect. These high levels of systemic cytokine can lead to severe toxicity and adverse reactions.

For use in therapy, it is therefore desirable to specifically deliver a signal transduction pathway factor, such as a cytokine, to a specific site in vivo (e.g. a tumor or tumor microenvironment in the case of cancer therapy). This can be achieved by conjugating the factor to a targeting moiety, e.g. an antibody or an antibody fragment, specific for the site. Early strategies aimed at delivering signal transduction pathway factors, such as cytokines, to a specific site in vivo included immunoglobulin heavy chains conjugated to various cytokines, including lymphotoxin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF) (reviewed e.g. in Lode et al., *Pharmacol Ther* 80, 277-292 (1998)). Researchers observed that, not only were they able to target cytokines to specific sites in vivo, they were also able to take advantage of the fact that monoclonal antibodies have longer serum half-lives than most other proteins. Given the systemic toxicity associated with high doses of certain unconjugated cytokines, e.g. IL-2, the ability of an immunoglobulin-cytokine fusion protein to maximize therapeutically beneficial biological activities at a desired site, e.g. in a tumor, whilst keeping systemic side effects to a minimum at a lower dose led researchers to believe that immunoglobulin-cytokine immunoconjugates were optimal therapeutic agents.

Nevertheless, there are certain disadvantages associated with the immunoglobulin-cytokine immunoconjugates

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known in the art. For example, these immunoconjugates have at least one cytokine coupled to each of the two immunoglobulin heavy chains, resulting in an immunoconjugate with bivalent target binding and two or more cytokine moieties (reviewed e.g. in Chang et al., *Expert Opin Drug Discovery* 4, 181-194 (2009), or Ortiz-Sanchez et al., *Expert Opin Biol Ther* 8, 609-632 (2008)). FIG. 1 depicts a conventional immunoglobulin-cytokine immunoconjugate as it is known in the art, where a cytokine is fused to the C-terminus of each of the two antibody heavy chains. Due to the presence of two or more cytokine moieties, such an immunoconjugate has a high avidity to the respective cytokine receptor (for example, picomolar affinity in the case of IL-2), and thus is targeted rather to the immune effector cells expressing the cytokine receptor than to the target antigen of the immunoglobulin (nM affinity) to which the cytokine is linked. Moreover, conventional immunoconjugates are known to be associated with infusion reactions (see e.g. King et al., *J Clin Oncol* 22, 4463-4473 (2004)), resulting at least partially from activation of cytokine receptors on immune effector cells in peripheral blood by the immunoconjugate's cytokine moieties.

Additionally, via their Fc domain, immunoglobulin-cytokine immunoconjugates can activate complement and interact with Fc receptors. This inherent immunoglobulin feature has been viewed unfavorably because therapeutic immunoconjugates may be targeted to cells expressing Fc receptors rather than the preferred antigen-bearing cells. Moreover, the simultaneous activation of cytokine receptors and Fc receptor signaling pathways leading to cytokine release, especially in combination with the long half-life of immunoglobulin fusion proteins, make their application in a therapeutic setting difficult due to systemic toxicity.

One approach to overcoming this problem is the use of immunoglobulin fragments devoid of an Fc domain, such as scFv or Fab fragments, in immunoconjugates. Examples of immunoglobulin fragment-cytokine immunoconjugates include the scFv-IL-2 immunoconjugate as set forth in PCT publication WO 2001/062298, the scFv-IL-12-scFv immunoconjugate as set forth in PCT publication WO 2006/119897 (wherein each of the two scFv fragments is connected to a subunit of the IL-12 heterodimer that is held together by disulfide bond(s)) or the Fab-IL-2-Fab immunoconjugates as set forth in PCT publication WO 2011/020783. Both the tumor-binding reactivity of the immunoglobulin parent molecule and the functional activity of the cytokine are maintained in most of these types of immunoconjugates, however the half-life of such constructs is considerably shorter than of immunoglobulin fusion proteins.

Therefore there remains a need for immunoconjugates with improved properties, for greater therapeutic effectiveness and a reduction in the number and severity of the side effects of these products (e.g., toxicity, destruction of non-tumor cells, etc.).

The present invention provides immunoglobulin-like immunoconjugates that exhibit improved efficacy, high specificity of action, reduced toxicity, and improved half-life and stability in blood relative to known immunoconjugates.

## SUMMARY OF THE INVENTION

The present invention is based, in part, on the inventors' recognition that immunoconjugates comprising more than one effector moiety, such as e.g. a cytokine, may be targeted to the respective effector moiety receptor rather than the target antigen of the antigen binding moiety of the immunoconjugate. Therefore, in one aspect the invention provides

an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. In one embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain, optionally through a linker peptide. In one embodiment the first antigen binding moiety is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain, optionally through a linker peptide or an immunoglobulin hinge region.

In one embodiment the first antigen binding moiety comprises an antigen binding domain of an antibody. In a particular embodiment the first antigen binding moiety is a Fab molecule. In certain embodiments the Fc domain comprises a modification promoting heterodimerization of two non-identical polypeptide chains. In a specific embodiment said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain. In a particular embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification.

In one embodiment the Fc domain is an IgG Fc domain, particularly an IgG<sub>1</sub> Fc domain. In a particular embodiment the Fc domain is human.

In certain embodiments of the invention the Fc domain is engineered to have altered binding to an Fc receptor, specifically altered binding to an Fcγ receptor, and/or altered effector function, specifically altered antibody-dependent cell-mediated cytotoxicity (ADCC).

Although the presence of an Fc domain is essential for prolonging the half-life of the immunoconjugate, the inventors realize that in some situations it will be beneficial to eliminate effector functions associated with engagement of Fc receptors by the Fc domain. Hence, in particular embodiments the altered binding to an Fc receptor and/or effector function is reduced binding and/or effector function. In a specific such embodiment the Fc domain comprises one or more amino acid mutation that reduces the binding of the Fc domain to an Fc receptor, particularly an Fcγ receptor. Preferably, such an amino acid mutation does not reduce binding to FcRn receptors. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a particular embodiment the Fc domain comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits.

On the other hand, there may be situations where it is desirable to enhance the effector functions of immunoconjugates. Hence, in certain embodiments the Fc domain of the immunoconjugate of the invention is engineered to have altered binding to an Fc receptor, specifically an Fcγ receptor, more specifically an FcγIIIa receptor, and/or altered effector function, wherein the altered binding and/or effector function is increased binding and/or effector function. In one such embodiment the Fc domain is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain. In a particular such embodiment the Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain. In a more specific embodiment the Fc domain comprises at least 20%, particularly at least 50%, more particularly at least 70% non-fucosylated oligosaccharides. In another specific embodiment the Fc domain comprises an increased proportion of bisected oligosaccharides, as compared to a non-engineered Fc domain. In yet another specific embodiment the Fc domain comprises an increased propor-

tion of bisected, non-fucosylated oligosaccharides, compared to a non-engineered Fc domain. In some embodiments said altered oligosaccharide structure results from increased β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity in a host cell used for expression of the immunoconjugate.

In a particular aspect, the invention provides immunoconjugates that comprise a first and a second antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. In one embodiment the first and the second antigen binding moiety and the Fc domain are part of an immunoglobulin molecule. In certain embodiments the immunoconjugate essentially consists of an immunoglobulin molecule and an effector moiety and optionally one or more linker sequences. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG<sub>1</sub> subclass immunoglobulin. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In a particular embodiment the immunoconjugate of the invention comprises an immunoglobulin molecule comprising two antigen binding moieties and an Fc domain, and an effector moiety fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, wherein not more than one effector moiety is present and wherein the Fc domain is engineered to have reduced binding to an Fc receptor, specifically altered binding to an Fcγ receptor, and/or reduced effector function.

In certain embodiments said first antigen binding moiety, or said first and said second antigen binding moiety, is directed to an antigen associated with a pathological condition, such as an antigen presented on a tumor cell or in a tumor cell environment, at a site of inflammation, or on a virus-infected cell. In a more specific embodiment said antigen is selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

In certain embodiments the effector moiety is a single chain effector moiety. In a particular embodiment the effector moiety is a cytokine. In one embodiment said cytokine is selected from the group of IL-2, IL-7, IL-10, IL-12, IL-15, IFN-α and IFN-γ. In a particular embodiment said cytokine is IL-2. In an even more particular embodiment said cytokine is a mutant IL-2 polypeptide having reduced binding affinity to the α-subunit of the IL-2 receptor. In a specific embodiment said mutant IL-2 polypeptide comprises an amino acid substitution at one or more positions selected from the positions corresponding to residues 42, 45 and 72 of human IL-2. In another particular embodiment the cytokine is IL-10. In yet another embodiment, the cytokine is IL-15, particularly a mutant IL-15 polypeptide having reduced binding affinity to the α-subunit of the IL-15 receptor. In another embodiment, the cytokine is IFN-α.

According to another aspect of the invention there is provided an isolated polynucleotide encoding an immunoconjugate of the invention or a fragment thereof. The invention further provides an expression vector comprising the isolated polynucleotide of the invention, and a host cell comprising the isolated polynucleotide or the expression vector of the invention. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell. In some

embodiments, the host cell has been manipulated to express increased levels of one or more polypeptides having  $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In one such embodiment the host cell has been further manipulated to express increased levels of one or more polypeptides having  $\alpha$ -mannosidase II (ManII) activity.

In another aspect is provided a method of producing the immunoconjugates of the invention, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the immunoconjugate and b) recovering the immunoconjugate. The invention also encompasses an immunoconjugate produced by the method of the invention.

The invention further provides a pharmaceutical composition comprising an immunoconjugate of the invention and a pharmaceutically acceptable carrier.

Also encompassed by the invention are methods of using the immunoconjugates and pharmaceutical compositions of the invention. In one aspect the invention provides an immunoconjugate or a pharmaceutical composition of the invention for use as a medicament. In one aspect is provided an immunoconjugate or a pharmaceutical composition according to the invention for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer. In other embodiments the disease is an inflammatory disorder. In a particular such embodiment the immunoconjugate comprises an IL-10 effector moiety.

Also provided is the use of an immunoconjugate of the invention for the manufacture of a medicament for the treatment of a disease in an individual in need thereof; as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the immunoconjugate according to the invention in a pharmaceutically acceptable form. In a specific embodiment the disease is cancer. In other embodiments the disease is an inflammatory disorder. In a particular such embodiment the immunoconjugate comprises an IL-10 effector moiety.

In any of the above embodiments the individual preferably is a mammal, particularly a human.

In a further aspect, the invention provides a conjugate comprising a first Fab molecule which does not specifically bind any antigen, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. In a particular embodiment the first Fab molecule comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297. In one embodiment, the effector moiety is fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain, optionally through a linker peptide. In another embodiment, the first Fab molecule is fused to the amino-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide or an immunoglobulin hinge region. In one embodiment, the conjugate comprises (i) an immunoglobulin molecule, comprising a first and a second Fab molecule which do not specifically bind any antigen and an Fc domain, and (ii) an effector moiety, wherein not more than one effector moiety is present. In one embodiment the immunoglobulin molecule is an IgG class immunoglobulin, particularly an IgG1 subclass immunoglobulin. In a particular embodiment the immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297. Specifically, the heavy chain variable region sequence of SEQ ID NO: 299 and the

light chain variable region sequence of SEQ ID NO: 297 are comprised in the first and the second Fab molecule of the immunoglobulin molecule. In one embodiment, the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In certain embodiments the Fc domain of the conjugate comprises a modification promoting heterodimerization of the non-identical polypeptide chains. In a specific embodiment, said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain. In a particular embodiment, the effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification. In one embodiment, the Fc domain is an IgG Fc domain, particularly an IgG<sub>1</sub> Fc domain. In a particular embodiment, the Fc domain is human. In some embodiments, the Fc domain is engineered to have altered binding to an Fc receptor, specifically altered binding to an Fc $\gamma$  receptor, and/or altered effector function, specifically altered ADCC. In some embodiments the Fc domain of the conjugate is engineered to have reduced binding to an Fc receptor, specifically reduced binding to an Fc $\gamma$  receptor, and/or reduced effector function, specifically reduced ADCC. In one embodiment, the Fc domain comprises one or more amino acid mutation that reduces the binding of the Fc domain to an Fc receptor, particularly an Fc $\gamma$  receptor. In a specific embodiment the amino acid mutation is an amino acid substitution at position P329. In a particular embodiment, the Fc domain of the conjugate comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits. In another embodiment of the conjugate of the invention, the Fc domain is engineered to have altered binding to an Fc receptor and/or altered effector function, wherein said altered binding and/or effector function is increased binding and/or effector function. In one embodiment of the conjugate of the invention, the Fc domain is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain. In a specific embodiment, the Fc domain described above comprises an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain.

In a further embodiment of the conjugate of the invention, the conjugate comprises a first and a second Fab molecule. In one embodiment, the first and the second Fab molecule each comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297. In one embodiment, the first and said second Fab molecule and said Fc domain are part of an immunoglobulin molecule. In a particular embodiment, the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment, the immunoglobulin molecule is an IgG<sub>1</sub> subclass immunoglobulin. In one embodiment, the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In some embodiments of the conjugate of the invention, said effector moiety is a single chain effector moiety. In one embodiment the effector moiety is a cytokine, particularly IL-2. In another embodiment, said cytokine is a mutant IL-2 polypeptide having reduced binding affinity to the  $\alpha$ -subunit of the IL-2 receptor. In a specific embodiment, said mutant IL-2 polypeptide comprises an amino acid substitution at one or more positions selected from the positions corresponding to residues 42, 45 and 72 of human IL-2.

Additionally, the conjugate can incorporate, alone or in combination, any of the features described herein in relation to the formats, the Fc domain or the effector moiety of the immunoconjugates of the invention.

The invention also provides an isolated polynucleotide encoding the conjugate of the invention of a fragment thereof, as described above. In a specific embodiment, the isolated polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 298 or SEQ ID NO: 300. The invention further provides an expression vector comprising the isolated polynucleotide, and a host cell comprising the isolated polynucleotide or the expression vector of the invention. In another aspect is provided a method of producing the conjugate of the invention described above, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the conjugate and b) recovering the conjugate. The invention also encompasses a conjugate, described above, produced by the method of the invention, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the conjugate and b) recovering the conjugate.

The invention further provides a pharmaceutical composition comprising the conjugate of the invention described above and a pharmaceutically acceptable carrier. Furthermore, the conjugate can be employed in the methods of use described herein for the immunoconjugates of the invention. In one embodiment, the conjugate as described above, or the pharmaceutical composition described above, is for use in the treatment of a disease in an individual in need thereof or for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

In a further aspect of the invention, a method of treating a disease in an individual is provided, comprising administering to said individual a therapeutically effective amount of a composition comprising the conjugate of the invention as described above, in a pharmaceutically acceptable form.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Schematic representation of typical immunoglobulin-cytokine immunoconjugate as known in the art, with a cytokine (dotted) fused to the C-terminus of each of the two immunoglobulin heavy chains.

FIG. 2. Schematic representation of novel immunoconjugates according to the invention, comprising not more than one effector moiety (dotted). The effector moiety is fused, optionally via a linker peptide (grey boxes) to the carboxy-terminal (format A and B) or the amino-terminal amino acid (format C) of the Fc domain. The immunoconjugate comprises one (format B and C) or more (typically two, format A) antigen binding moieties, which may be Fab fragments comprising antibody heavy and light chain variable domains (hatched). The Fc domain may comprise a modification promoting heterodimerization of two non-identical polypeptide chains (black dot) and/or a modification altering Fc receptor binding and/or effector function (black star).

FIG. 3. Purification of FAP-targeted 4G8-based IgG-IL-2 quadruple mutant (qm) immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (97% monomer content).

FIG. 4. Purification of FAP-targeted 28H1-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (reduced: NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer; non-reduced: NuPAGE Tris-Acetate, Invitrogen, Tris-Acetate running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIG. 5. Purification of FAP-targeted 28H1-based IgG-IL-2 qm immunoconjugate from CHO cells. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIG. 6. Purification of FAP-targeted 4B9-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIG. 7. Purification of CEA-targeted CH1A1A 98/99 2F1-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.8% monomer content).

FIG. 8. Purification of TNC A2-targeted 2B10-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (100% monomer content).

FIG. 9. Purification of untargeted DP47GS-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIG. 10. Binding of FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate to human FAP expressed on stably transfected HEK 293 cells as measured by FACS, compared to the corresponding Fab-IL-2 qm-Fab construct.

FIG. 11. Interferon (IFN)- $\gamma$  release on NK92 cells induced by FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate in solution, compared to the 28H1-based Fab-IL-2 qm-Fab construct.

FIG. 12. Detection of phosphorylated STATS by FACS in different cell types after stimulation for 20 min with FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate in solution, compared to the 28H1-based Fab-IL-2-Fab and Fab-IL-2 qm-Fab constructs as well as Proleukin. A) NK cells (CD3<sup>+</sup>CD56<sup>+</sup>); B) CD8<sup>+</sup> T cells (CD3<sup>+</sup>CD8<sup>+</sup>); C) CD4<sup>+</sup> T cells (CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>-</sup>CD127<sup>+</sup>); D) regulatory T cells (CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup>).

FIG. 13. Binding of TNC A2-targeted 2B10 IgG-IL-2 qm and corresponding unconjugated IgG to TNC A2-expressing U87MG cells, as measured by FACS.

FIG. 14. Induction of NK92 cell proliferation by TNC A2-targeted 2B10 IgG-IL-2 qm, CEA-targeted CH1A1A 98/99 2F1 IgG-IL-2 qm and CH1A1A 98/99 2F1 IgG-IL-2 wt immunoconjugates.

FIG. 15. Induction of NK92 cell proliferation by FAP-targeted 4B9 IgG-IL-2 qm and 4B9 IgG-IL-2 wt immunoconjugates.

FIG. 16. Killing (as measured by LDH release) of CEA-overexpressing A549 tumor cells by PBMCs through ADCC mediated by glycoengineered (ge) and wildtype (wt) CH1A1A IgG-IL-2 qm immunoconjugates, compared to unconjugated glycoengineered CH1A1A IgG.

FIG. 17. Purification of untargeted DP47GS IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (99.6% monomer content).

FIG. 18. Purification of 28H1-based FAP-targeted 28H1 IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (99.6% monomer content).

FIG. 19. Purification of CEA-targeted CH1A1A 98/99 2F1-based IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (100% monomer content).

FIG. 20. Purification of FAP-targeted 4B9-based IgG-IL-2 wt immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Zoom on the elution profile of the size exclusion chromatography step in A. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.5% monomer content).

FIG. 21. A) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel (Invitrogen), NuPAGE LDS sample buffer (4 $\times$ ), heated for 10 min at 70° C., MOPS buffer, 160 V, 60 min, MW marker Mark 12, unstained standard (Invitrogen, M) of reduced (1) and non-reduced (2) 2B10 IgG-IL-10M1. B) SPR-based affinity determination (ProteOn XPR36) of 2B10 IgG-IL-10M1 to human TNC A2 fitted globally to a 1:1 interaction model.

(chip: NLC; ligand: TNCA2 (250 RU); analyte: TNCA2 2B10 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180 s; dissociation time: 600 s; flow rate: 50  $\mu$ l/min;  $k_{on}$  1.80 $\times 10^6$  l/Ms;  $k_{off}$  9.35 $\times 10^{-5}$  l/s;  $K_D$ : 52 pM). C) SPR-based affinity determination (ProteOn XPR36) of 2B10 IgG-IL-10M1 to human IL-10R1 fitted globally to a 1:1 interaction model (chip: NLC; ligand: IL-10R1 (1600RU); analyte: TNCA2 2B10 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180 s; disso-

ciation time: 600 s; flow rate: 50  $\mu$ l/min;  $k_{on}$  5.56 $\times 10^5$  l/Ms;  $k_{off}$  2.89 $\times 10^{-4}$  l/s;  $K_D$ : 520 pM).

FIG. 22. A) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel (Invitrogen), NuPAGE LDS sample buffer (4 $\times$ ), heated for 10 min at 70° C., MOPS buffer, 160 V, 60 min, MW marker Mark 12, unstained standard (Invitrogen, M) of reduced (1) and non-reduced (2) 4G8 IgG-IL-10M1. B) SPR-based affinity determination (ProteOn XPR36) of 4G8 IgG-IL-10M1 to human FAP fitted globally to a 1:1 interaction model (chip: GLM; ligand: huFAP (500RU); analyte: FAP 4G8 IgG-IL-10M1 164 kDa; concentration range analyte: 10, 2, 0.4, 0.08, 0 nM; association time: 180 s; dissociation time: 600 s; flow rate: 50  $\mu$ l/min;  $k_{on}$  6.68 $\times 10^5$  l/Ms;  $k_{off}$  1.75 $\times 10^{-5}$  l/s;  $K_D$ : 26 pM). C) SPR-based affinity determination (ProteOn XPR36) of 4G8 IgG-IL-10M1 to human IL-10R1 fitted globally to a 1:1 interaction model (chip: NLC; ligand: IL 10R1 (1600RU); analyte: FAP 4G8 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180 s; dissociation time: 600 s; flow rate: 50  $\mu$ l/min;  $k_{on}$  3.64 $\times 10^5$  l/Ms;  $k_{off}$  2.96 $\times 10^{-4}$  l/s;  $K_D$ : 815 pM).

FIG. 23. Purification of FAP-targeted 4B9-based "1+1" IgG-IL-2 qm immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (99.2% monomer content).

FIG. 24. Purification of FAP-targeted 28H1-based "1+1" IgG-IL-2 qm immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (100% monomer content).

FIG. 25. Purification of FAP-targeted 4B9-based "1+1" IgG-IL-7 immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical capillary electrophoresis SDS (Caliper) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.6% monomer content).

FIG. 26. Purification of FAP-targeted 4B9-based "1+1" IgG-IFN- $\alpha$  immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (92.8% monomer content).

FIG. 27. Induction of NK92 cell proliferation by FAP-targeted 4B9 "1+1" IgG-IL-2 qm and 28H1 "1+1" IgG-IL-2 wt immunoconjugates, compared to corresponding IgG-IL-2 constructs.

FIG. 28. Proliferation of PHA-activated (A) CD4 and (B) CD8 T cells induced by 4B9 "1+1" IgG-IL-7 and 4B9 "1+1" IgG-IL-2 qm immunoconjugates, compared to IgG-IL-2 qm and IgG-IL-2 wt constructs.

FIG. 29. Induction of Daudi cell proliferation by 4B9 "1+1" IgG-IFN- $\alpha$ , compared to Roferon A.

FIG. 30. Serum concentrations of IL-2 immunoconjugates after a single i.v. administration of FAP-targeted (A) and untargeted (B) IgG-IL-2 constructs comprising either wild-type (wt) or quadruple mutant (qm) IL-2.

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FIG. 31. Tissue distribution of FAP-targeted 28H1 IgG-IL qm compared to unconjugated FAP-targeted 28H1 IgG and 4B9 IgG, as well as untargeted DP47GS IgG, 24 hours after i.v. injection.

FIG. 32. Binding of 28H1 IgG-IL-2 qm and 28H1 IgG-(IL-2 qm)<sub>2</sub> immunoconjugates to NK92 cells as determined by FACS.

FIG. 33. Proliferation of NK cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

FIG. 34. Proliferation of CD4 T-cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

FIG. 35. Proliferation of CD8 T-cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

FIG. 36. Proliferation of pre-activated CD8 (A) and CD4 (B) T cells after six days incubation with different IL-2 immunoconjugates.

FIG. 37. Activation induced cell death of CD3<sup>+</sup> T cells after six days incubation with different IL-2 immunoconjugates and overnight treatment with anti-Fas antibody.

FIG. 38. Serum concentrations of IL-2 immunoconjugates after a single i.v. administration of untargeted DP47GS IgG-IL-2 constructs comprising either wild-type (A) or quadruple mutant IL-2 (B).

FIG. 39. Binding of DP47GS IgG to different antigens. Binding was detected in an ELISA-based assay with the antigens captured on the plate. A human IgG1 antibody which exhibits unspecific binding to almost all of the captured antigens was used as positive control, blank samples did not contain any antibody.

FIG. 40. Binding of DP47GS IgG with or without LALA P329G mutation in the Fc domain to subsets of fresh (A), PHA-L activated (B) and re-stimulated (C) human PBMCs, as determined by FACS analysis. Upper left panel: B cells (in A, B) or CD4<sup>+</sup> T cells (in C); upper right panel: CD8<sup>+</sup> T cells; lower left panel: NK cells; lower right panel: CD14<sup>+</sup> cells (monocytes/neutrophils).

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

Terms are used herein as generally used in the art, unless otherwise defined in the following.

As used herein, the term “conjugate” refers to a fusion polypeptide molecule that includes one effector moiety and a further peptide molecule, particularly an immunoglobulin molecule.

As used herein, the term “immunoconjugate” refers to a fusion polypeptide molecule that includes one effector moiety, at least one antigen binding moiety and an Fc domain, provided that not more than one effector moiety is present. In certain embodiments, the immunoconjugate comprises one effector moiety, two antigen binding moieties, and an Fc domain. Particular immunoconjugates according to the invention essentially consist of one effector moiety, two antigen binding moieties, and an Fc domain, joined by one or more linker sequences. The antigen binding moiety and the effector moiety can be joined to the Fc domain by a variety of interactions and in a variety of configurations as described herein. In a particular embodiment, the two antigen binding moieties and the Fc domain are joined to each other in a configuration so as to form a full immunoglobulin molecule. An immunoconjugate as referred to herein, is a

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fusion protein, i.e. the components of the immunoconjugate are linked to each other by peptide-bonds, either directly or through linker peptides.

As used herein, the term “antigen binding moiety” refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one embodiment, an antigen binding moiety is able to direct the entity to which it is attached (e.g. an effector moiety or a second antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant. Antigen binding moieties include antibodies and fragments thereof as further defined herein. Particular antigen binding moieties include an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In certain embodiments, the antigen binding moieties may comprise antibody constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes:  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , or  $\mu$ . Useful light chain constant regions include any of the two isotypes:  $\kappa$  and  $\lambda$ .

As used herein, the term “antigenic determinant” is synonymous with “antigen” and “epitope,” and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, free in blood serum, and/or in the extracellular matrix (ECM). In a particular embodiment the antigenic determinant is a human antigen.

By “specifically binds” is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen-binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain embodiments, an antigen binding moiety that binds to the antigen, or an immunoconjugate comprising that antigen binding moiety, has a dissociation constant ( $K_D$ ) of  $\leq 1 \mu\text{M}$ ,  $\leq 100 \text{ nM}$ ,  $\leq 10 \text{ nM}$ ,  $\leq 1 \text{ nM}$ ,  $\leq 0.1 \text{ nM}$ ,  $\leq 0.01 \text{ nM}$ , or  $\leq 0.001 \text{ nM}$  (e.g.  $10^{-8} \text{ M}$  or less, e.g. from  $10^{-8} \text{ M}$  to  $10^{-13} \text{ M}$ , e.g., from  $10^{-9} \text{ M}$  to  $10^{-13} \text{ M}$ ).

“Affinity” refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., receptor and a ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ( $K_D$ ), which is the ratio of dissociation and association rate constants ( $k_{\text{off}}$  and  $k_{\text{on}}$ , respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well established methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

“Reduced binding”, for example reduced binding to an Fc receptor or to CD25, refers to a decrease in affinity for the respective interaction, as measured for example by SPR. For clarity the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete abolishment of the interaction. Conversely, “increased binding” refers to an increase in binding affinity for the respective interaction.

As used herein, the terms “first” and “second” with respect to antigen-binding moieties etc., are used for convenience of distinguishing when there is more than one of each type of moiety. Use of these terms is not intended to confer a specific order or orientation of the immunoconjugate unless explicitly so stated.

As used herein, the term “effector moiety” refers to a polypeptide, e.g., a protein or glycoprotein, that influences cellular activity, for example, through signal transduction or other cellular pathways. Accordingly, the effector moiety of the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response in a cell bearing one or more receptors for the effector moiety. In one embodiment, an effector moiety can elicit a cytotoxic response in cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit a proliferative response in cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit differentiation in cells bearing receptors for the effector moiety. In another embodiment, an effector moiety can alter expression (i.e. upregulate or downregulate) of an endogenous cellular protein in cells bearing receptors for the effector moiety. Non-limiting examples of effector moieties include cytokines, growth factors, hormones, enzymes, substrates, and cofactors. The effector moiety can be associated with an antigen-binding moiety or an Fc domain in a variety of configurations to form an immunoconjugate.

As used herein, the term “cytokine” refers to a molecule that mediates and/or regulates a biological or cellular function or process (e.g. immunity, inflammation, and hematopoiesis). The term “cytokine” as used herein includes “lymphokines,” “chemokines,” “monokines,” and “interleukins”. Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , MIP-1 $\alpha$ , MIP-1 $\beta$ , TGF- $\beta$ , TNF- $\alpha$ , and TNF- $\beta$ . Particular cytokines are IL-2, IL-7, IL-10, IL-12, IL-15, IFN- $\alpha$  and IFN- $\gamma$ . In particular embodiments the cytokine is a human cytokine. The term “cytokine” as used herein is meant to also include cytokine variants comprising one or more amino acid mutations in the amino acid sequences of the corresponding wild-type cytokine, such as for example the IL-2 variants described in Sauv   et al., *Proc Natl Acad Sci USA* 88, 4636-40 (1991); Hu et al., *Blood* 101, 4853-4861 (2003) and US Pat. Publ. No. 2003/0124678; Shanafelt et al., *Nature Biotechnol* 18, 1197-1202 (2000); Heaton et al., *Cancer Res* 53, 2597-602 (1993) and U.S. Pat. No. 5,229,109; US Pat. Publ. No. 2007/0036752; WO 2008/0034473; WO 2009/061853; or PCT patent application no. PCT/EP2012/051991. Further cytokine variants, for example variants of IL-15, are described herein. In certain embodiments cytokines have been mutated to eliminate glycosylation.

As used herein, the term “single-chain” refers to a molecule comprising amino acid monomers linearly linked by peptide bonds. In one embodiment, the effector moiety is a single-chain effector moiety. Non-limiting examples of single-chain effector moieties include cytokines, growth

factors, hormones, enzymes, substrates, and cofactors. When the effector moiety is a cytokine and the cytokine of interest is normally found as a multimer in nature, each subunit of the multimeric cytokine is sequentially encoded by the single-chain of the effector moiety. Accordingly, non-limiting examples of useful single-chain effector moieties include GM-CSF, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , MIP-1 $\alpha$ , MIP-1 $\beta$ , TGF- $\beta$ , TNF- $\alpha$ , and TNF- $\beta$ .

As used herein, the term “control effector moiety” refers to an unconjugated effector moiety. For example, when comparing an IL-2 immunoconjugate as described herein with a control effector moiety, the control effector moiety is free, unconjugated IL-2. Likewise, e.g., when comparing an IL-12 immunoconjugate with a control effector moiety, the control effector moiety is free, unconjugated IL-12 (e.g. existing as a heterodimeric protein wherein the p40 and p35 subunits share only disulfide bond(s)).

As used herein, the term “effector moiety receptor” refers to a polypeptide molecule capable of binding specifically to an effector moiety. For example, where IL-2 is the effector moiety, the effector moiety receptor that binds to an IL-2 molecule (e.g. an immunoconjugate comprising IL-2) is the IL-2 receptor. Similarly, e.g., where IL-12 is the effector moiety of an immunoconjugate, the effector moiety receptor is the IL-12 receptor. Where an effector moiety specifically binds to more than one receptor, all receptors that specifically bind to the effector moiety are “effector moiety receptors” for that effector moiety.

The term “immunoglobulin molecule” refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N— to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called  $\alpha$  (IgA),  $\delta$  (IgD),  $\epsilon$  (IgE),  $\gamma$  (IgG), or  $\mu$  (IgM), some of which may be further divided into subtypes, e.g.  $\gamma_1$  (IgG<sub>1</sub>),  $\gamma_2$  (IgG<sub>2</sub>),  $\gamma_3$  (IgG<sub>3</sub>),  $\gamma_4$  (IgG<sub>4</sub>),  $\alpha_1$  (IgA<sub>1</sub>) and  $\alpha_2$  (IgA<sub>2</sub>). The light chain of an immunoglobulin may be assigned to one of two types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, and antibody fragments so long as they exhibit the desired antigen-binding activity.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies. For a review of certain antibody fragments, see Hudson et al., *Nat Med* 9, 129-134 (2003). For a review of scFv fragments, see e.g. Pl  ckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg

and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')<sub>2</sub> fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Pat. No. 5,869, 046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see e.g. U.S. Pat. No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

The term "antigen binding domain" refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., *Kuby Immunology*, 6<sup>th</sup> ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as "complementarity determining regions" (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, *Sequences of Proteins of Immunological Interest* (1983) and by Chothia et al., J Mol Biol 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a

comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1

CDR Definitions <sup>1</sup>			
CDR	Kabat	Chothia	AbM <sup>2</sup>
V <sub>H</sub> CDR1	31-35	26-32	26-35
V <sub>H</sub> CDR2	50-65	52-58	50-58
V <sub>H</sub> CDR3	95-102	95-102	95-102
V <sub>L</sub> CDR1	24-34	26-32	24-34
V <sub>L</sub> CDR2	50-56	50-52	50-56
V <sub>L</sub> CDR3	89-97	91-96	89-97

<sup>1</sup>Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

<sup>2</sup>"AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat et al. also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

The polypeptide sequences of the sequence listing (i.e., SEQ ID NOs 23, 25, 27, 29, 31, etc.) are not numbered according to the Kabat numbering system. However, it is well within the ordinary skill of one in the art to convert the numbering of the sequences of the Sequence Listing to Kabat numbering.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2 (L2)-FR3-H3 (L3)-FR4.

The "class" of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

The term "Fc domain" or "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health,

Bethesda, Md., 1991. A "subunit" of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

A "modification promoting heterodimerization" is a manipulation of the peptide backbone or the post-translational modifications of a polypeptide that reduces or prevents the association of the polypeptide with an identical polypeptide to form a homodimer. A modification promoting heterodimerization as used herein particularly includes separate modifications made to each of two polypeptides desired to form a dimer, wherein the modifications are complementary to each other so as to promote association of the two polypeptides. For example, a modification promoting heterodimerization may alter the structure or charge of one or both of the polypeptides desired to form a dimer so as to make their association sterically or electrostatically favorable, respectively. Heterodimerization occurs between two non-identical polypeptides, such as two subunits of an Fc domain wherein further immunoconjugate components fused to each of the subunits (e.g. antigen binding moiety, effector moiety) are not the same. In the immunoconjugates according to the present invention, the modification promoting heterodimerization is in the Fc domain. In some embodiments the modification promoting heterodimerization comprises an amino acid mutation, specifically an amino acid substitution. In a particular embodiment, the modification promoting heterodimerization comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

The term "effector functions" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

As used herein, the terms "engineer, engineered, engineering", are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches. "Engineering", particularly with the prefix "glyco-", as well as the term "glycosylation engineering" includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity. Glycosylation engineering can be used to obtain a "host cell having increased GnTIII activity", a "host cell having increased ManII activity", or a "host cell having decreased  $\alpha(1,6)$  fucosyltransferase activity".

The term "amino acid mutation" as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., reduced binding to an Fc receptor, or reduced binding to CD25. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. Particular amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an Fc region or a cytokine such as IL-2, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are particularly preferred. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution from proline at position 329 of the Fc domain to glycine can be indicated as 329G, G329, G<sub>329</sub>, P329G, or Pro329Gly.

As used herein, term "polypeptide" refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis. A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

By an "isolated" polypeptide or a variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are con-

sidered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "polynucleotide" refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA). The term "nucleic acid molecule" refers to any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By "isolated" nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a

recombinant polynucleotide encoding a therapeutic polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extra-chromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette of the invention comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "vector" or "expression vector" is synonymous with "expression construct" and refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated in a target cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression

vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "artificial" refers to a synthetic, or non-host cell derived composition, e.g. a chemically-synthesized oligonucleotide.

The terms "host cell", "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the immunoconjugates used for the present invention. In one embodiment, the host cell is engineered to allow the production of an immunoconjugate with modified oligosaccharides in its Fc region. In certain embodiments, the host cells have been manipulated to express increased levels of one or more polypeptides having  $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In certain embodiments the host cells have been further manipulated to express increased levels of one or more polypeptides having  $\alpha$ -mannosidase II (ManII) activity. Host cells include cultured cells, e.g. mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

As used herein, the term "polypeptide having GnTIII activity" refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in  $\beta$ -1,4 linkage to the  $\beta$ -linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of  $\beta(1,4)$ -N-acetylglucosaminyltransferase III, also known as  $\beta$ -1,4-mannosylglycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependency in a given activity as compared to the GnTIII (i.e. the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about ten-fold less activity, and most preferably, not more than about three-fold less activity relative to the GnTIII). In certain embodiments the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, the Golgi localization domain is the localization domain of mannosidase II or GnTI, most particularly the localization domain of mannosidase II. Alternatively, the Golgi localization domain is selected from the group consisting of: the localization

domain of mannosidase I, the localization domain of GnTII, and the localization domain of  $\alpha$ 1,6 core fucosyltransferase. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in WO 2004/065540, U.S. Provisional Pat. Appl. No. 60/495,142 and U.S. Pat. Appl. Publ. No. 2004/0241817, the entire contents of which are expressly incorporated herein by reference.

As used herein, the term "Golgi localization domain" refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide to a location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an enzyme.

As used herein, the term "polypeptide having ManII activity" refers to polypeptides that are able to catalyze the hydrolysis of the terminal 1,3- and 1,6-linked  $\alpha$ -D-mannose residues in the branched GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub> mannose intermediate of N-linked oligosaccharides. This includes polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of Golgi  $\alpha$ -mannosidase II, also known as mannosyl oligosaccharide 1,3-1,6- $\alpha$ -mannosidase II (EC 3.2.1.114), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB).

An "activating Fc receptor" is an Fc receptor that following engagement by an Fc region of an antibody (or immunoconjugate) elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include Fc $\gamma$ RIIIa (CD16a), Fc $\gamma$ RI (CD64), Fc $\gamma$ RIIa (CD32), and Fc $\alpha$ RI (CD89).

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies, immunoconjugates or fragments thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. As used herein, the term "increased ADCC" is defined as either an increase in the number of target cells that are lysed in a given time, at a given concentration of immunoconjugate in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or a reduction in the concentration of immunoconjugate, in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The increase in ADCC is relative to the ADCC mediated by the same immunoconjugate produced by the same type of host cells, using the same standard production, purification, formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example the increase in ADCC mediated by an immunoconjugate produced by host cells engineered to have an altered pattern of glycosylation (e.g. to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein, is relative to the ADCC mediated by the same immunoconjugate produced by the same type of non-engineered host cells.

By "immunoconjugate having increased antibody dependent cell-mediated cytotoxicity (ADCC)" is meant an immunoconjugate having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted in vitro ADCC assay is as follows:

- 1) the assay uses target cells that are known to express the target antigen recognized by the antigen binding moiety of the immunoconjugate;

- 2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;
- 3) the assay is carried out according to following protocol:
  - i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at  $5 \times 10^6$  cells/ml in RPMI cell culture medium;
  - ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of  $^{51}\text{Cr}$ , washed twice with cell culture medium, and resuspended in cell culture medium at a density of  $10^5$  cells/ml;
  - iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;
  - iv) the immunoconjugate is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting immunoconjugate solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various immunoconjugate concentrations covering the whole concentration range above;
  - v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the immunoconjugate solution (point iv above);
  - vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the immunoconjugate solution (point iv above);
  - vii) the 96-well microtiter plate is then centrifuged at  $50 \times g$  for 1 minute and incubated for 1 hour at  $4^\circ \text{C}$ ;
  - viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5%  $\text{CO}_2$  atmosphere at  $37^\circ \text{C}$  for 4 hours;
  - ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;
  - x) the percentage of specific lysis is calculated for each immunoconjugate concentration according to the formula  $(\text{ER}-\text{MR})/(\text{MR}-\text{SR}) \times 100$ , where ER is the average radioactivity quantified (see point ix above) for that immunoconjugate concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);
- 4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the immunoconjugate concentration range tested above, and/or a reduction in the concentration of immunoconjugate required to achieve one half of the maximum percentage of specific lysis observed within the immunoconjugate concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same immunoconjugate, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been engineered.

An "effective amount" of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

A "therapeutically effective amount" of an agent, e.g. a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). Particularly, the individual or subject is a human.

The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, immunoconjugates of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

#### DETAILED DESCRIPTION OF THE EMBODIMENTS

In a first aspect the invention provides an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. The absence of further effector moieties may reduce targeting of the immunoconjugate to sites where the respective effector moiety receptor is presented, thereby improving targeting to and accumulation at sites where the actual target antigen of the immunoconjugate, which is recognized by the antigen binding moiety, is presented. Furthermore, the absence of an avidity effect for the respective effector moiety receptor can reduce activation of effector moiety receptor-positive cells in peripheral blood upon intravenous administration of the immunoconjugate. Furthermore, the serum half-life of immunoconjugates comprising only a single effector moiety

appears to be longer as compared to immunoconjugates comprising two or more effector moieties.  
Immunoconjugate Formats

The components of the immunoconjugate can be fused to each other in a variety of configurations. Exemplary configurations are depicted in FIG. 2. In one embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the two subunits of the Fc domain. The effector moiety may be fused to the Fc domain directly or through a linker peptide, comprising one or more amino acids, typically about 2-20 amino acids. Linker peptides are known in the art or are described herein. Suitable, non-immunogenic linker peptides include, for example,  $(G_4S)_n$ ,  $(SG_4)_n$  or  $G_4(SG_4)_n$  linker peptides. "n" is generally a number between 1 and 10, typically between 2 and 4. Alternatively, where the effector moiety is linked to the N-terminus of an Fc domain subunit, it may be linked via an immunoglobulin hinge region or a portion thereof, with or without an additional linker peptide.

Similarly, the first antigen binding moiety can be fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain. In one embodiment the first antigen binding moiety is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain. The first antigen binding moiety may be fused to the Fc domain directly or through a linker peptide. In a particular embodiment the first antigen binding moiety is fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region.

In one embodiment the first antigen binding moiety comprises an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In a particular embodiment the first antigen binding moiety is a Fab molecule. In one embodiment the Fab molecule is fused at its heavy or light chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a particular embodiment the Fab molecule is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a more particular embodiment the Fab molecule is fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region.

In one embodiment the immunoconjugate essentially consists of an antigen binding moiety, an Fc domain consisting of two subunits, an effector moiety, and optionally one or more linker peptides, wherein said antigen binding domain is a Fab molecule and is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain, and wherein said effector moiety is fused either (i) to the amino-terminal amino acid of the other one of the two subunits of the Fc domain, or (ii) to the carboxy-terminal amino acid of one of the two subunits of the Fc domain. In the latter case, the effector moiety and the first antigen binding moiety may both be fused to the same subunit of the Fc domain, or may each be fused to a different one of the two subunits of the Fc domain.

An immunoconjugate format with a single antigen binding moiety (for example as shown in FIGS. 2B and 2C) is useful, particularly in cases where internalization of the target antigen is to be expected following binding of a high affinity antigen binding moiety. In such cases, the presence

of more than one antigen binding moiety per immunoconjugate may enhance internalization, thereby reducing availability of the target antigen.

In many other cases, however, it will be advantageous to have an immunoconjugate comprising two or more antigen binding moieties and a single effector moiety to optimize targeting to the target antigen versus the effector moiety receptor, and the pharmaceutical window of the immunoconjugate.

Thus, in a particular embodiment the immunoconjugate of the invention comprises a first and a second antigen binding moiety. In one embodiment each of said first and second antigen binding moieties is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain. The first and second antigen binding moieties may be fused to the Fc domain directly or through a linker peptide. In a particular embodiment each of said first and second antigen binding moieties is fused to a subunit of the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region.

In one embodiment each of said first and second antigen binding moieties comprises an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In a particular embodiment each of said first and second antigen binding moieties is a Fab molecule. In one embodiment each of said Fab molecules is fused at its heavy or light chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a particular embodiment each of said Fab molecules is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a more particular embodiment each of said Fab molecules is fused to a subunit of the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region.

In one embodiment the first and the second antigen binding moiety and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG<sub>1</sub> subclass immunoglobulin. In another particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains. The effector moiety may be fused to the immunoglobulin heavy chain directly or through a linker peptide. In a particular embodiment the immunoconjugate essentially consists of an immunoglobulin molecule, an effector moiety fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, and optionally one or more linker peptides.

In one embodiment the immunoconjugate comprises a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit and a polypeptide wherein an Fc domain subunit shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In another embodiment, the immunoconjugate comprises a polypeptide wherein a first Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, and a polypeptide wherein a second Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, which in turn shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In a further embodiment the immunoconjugate comprises a polypeptide wherein a Fab

heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit and a polypeptide wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit. In some embodiments the immunoconjugate further comprises a Fab light chain polypeptide. In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

According to any of the above embodiments, components of the immunoconjugate (e.g. effector moiety, antigen binding moiety, Fc domain) may be linked directly or through various linkers, particularly peptide linkers comprising one or more amino acids, typically about 2-20 amino acids, that are described herein or are known in the art. Suitable, non-immunogenic linker peptides include, for example, (G4S)<sub>n</sub>, (SG4)<sub>n</sub> or G4(SG4)<sub>n</sub> linker peptides, wherein n is generally a number between 1 and 10, typically between 2 and 4.

#### Fc Domain

The Fc domain of the immunoconjugate consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other. In one embodiment the immunoconjugate of the invention comprises not more than one Fc domain.

In one embodiment according to the invention the Fc domain of the immunoconjugate is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG1 Fc domain. In another embodiment, the Fc domain is an IgG4 Fc domain. In a further particular embodiment the Fc domain is human. An exemplary sequence of a human IgG1 Fc region is given in SEQ ID NO: 1.

The Fc domain confers to the immunoconjugate a greatly prolonged serum-half life as compared to immunoconjugate formats lacking an Fc domain. Particularly when the immunoconjugate comprises an effector moiety of rather weak activity (but e.g. reduced toxicity), a long half-life might be essential to achieve optimal efficacy in vivo. Moreover, the Fc domain can mediate effector functions, as will be further discussed below.

#### Fc Domain Modifications Promoting Heterodimerization

Immunoconjugates according to the invention comprise only one single effector moiety, fused to one of the two subunits of the Fc domain, thus they comprise two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides, out of which only heterodimers of the two non-identical polypeptides are useful according to the invention. To improve the yield and purity of immunoconjugates in recombinant production, it can thus be advantageous to introduce in the Fc domain of the immunoconjugate a modification which hinders the formation of homodimers of two identical polypeptides (i.e. two polypeptides comprising an effector moiety, or two polypeptides lacking an effector moiety) and/or promotes the formation of heterodimers of a polypeptide comprising an effector moiety and a polypeptide lacking an effector moiety.

Accordingly, in certain embodiments according to the invention the Fc domain of the immunoconjugate comprises a modification promoting heterodimerization of two non-identical polypeptide chains. The site of most extensive protein-protein interaction between the two polypeptide chains of a human IgG Fc domain is in the CH3 domain of

the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

In a specific embodiment said modification is a knob-into-hole modification, comprising a knob modification in one of the two subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain.

The knob-into-hole technology is described e.g. in U.S. Pat. No. 5,731,168; U.S. Pat. No. 7,695,936; Ridgway et al., *Prot Eng* 9, 617-621 (1996) and Carter, *J Immunol Meth* 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment a knob modification comprises the amino acid substitution T366W in one of the two subunits of the Fc domain, and the hole modification comprises the amino acid substitutions T366S, L368A and Y407V in the other one of the two subunits of the Fc domain. In a further specific embodiment, the subunit of the Fc domain comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc domain comprising the hole modification additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in formation of a disulfide bridge between the two subunits of the Fc region, further stabilizing the dimer (Carter, *J Immunol Methods* 248, 7-15 (2001)).

In an alternative embodiment a modification promoting heterodimerization of two non-identical polypeptide chains comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two polypeptide chains by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

In a particular embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification. Without wishing to be bound by theory, fusion of the effector moiety to the knob-containing subunit of the Fc domain will further minimize the generation of homodimeric immunoconjugates comprising two effector moieties (steric clash of two knob-containing polypeptides).

#### Fc Domain Modifications Altering Fc Receptor Binding

In certain embodiments of the invention the Fc domain of the immunoconjugate is engineered to have altered binding affinity to an Fc receptor, specifically altered binding affinity to an Fcγ receptor, as compared to a non-engineered Fc domain.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is

described herein. Alternatively, binding affinity of Fc domains or immunoconjugates comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as NK cells expressing FcγIIIa receptor.

In some embodiments the Fc domain of the immunoconjugate is engineered to have altered effector functions, particularly altered ADCC, as compared to a non-engineered Fc domain.

Effector function of an Fc domain, or an immunoconjugate comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom et al. *Proc Natl Acad Sci USA* 83, 7059-7063 (1986) and Hellstrom et al., *Proc Natl Acad Sci USA* 82, 1499-1502 (1985); U.S. Pat. No. 5,821,337; Bruggemann et al., *J Exp Med* 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g. in a animal model such as that disclosed in Clynes et al., *Proc Natl Acad Sci USA* 95, 652-656 (1998).

In some embodiments binding of the Fc domain to a complement component, specifically to C1q, is altered. Accordingly, in some embodiments wherein the Fc domain is engineered to have altered effector function, said altered effector function includes altered CDC. C1q binding assays may be carried out to determine whether the immunoconjugate is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J Immunol Methods* 202, 163 (1996); Cragg et al., *Blood* 101, 1045-1052 (2003); and Cragg and Glennie, *Blood* 103, 2738-2743 (2004)).

a) Decreased Fc Receptor Binding and/or Effector Function

The Fc domain confers to the immunoconjugate favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the immunoconjugate to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with the effector moiety and the long half-life of the immunoconjugate, results in excessive activation of cytokine receptors and severe side effects upon systemic administration. In line with this, conventional IgG-IL-2 immunoconjugates have been described to be associated with infusion reactions (see e.g. King et al., *J Clin Oncol* 22, 4463-4473 (2004)).

Accordingly, in particular embodiments according to the invention the Fc domain of the immunoconjugate is engineered to have reduced binding affinity to an Fc receptor. In one such embodiment the Fc domain comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment said amino acid

mutation reduces the binding affinity of the Fc domain to the Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to the Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the immunoconjugate comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to an immunoconjugate comprising a non-engineered Fc domain. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an Fcγ receptor, more specifically an FcγRIIIa, FcγRI or FcγRIIa receptor. Preferably, binding to each of these receptors is reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the immunoconjugate comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the immunoconjugate comprising said non-engineered form of the Fc domain) to FcRn. Fc domains, or immunoconjugates of the invention comprising said Fc domains, may exhibit greater than about 80% and even greater than about 90% of such affinity. In one embodiment the amino acid mutation is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is P329A or P329G, particularly P329G. In one embodiment the Fc domain comprises a further amino acid substitution at a position selected from S228, E233, L234, L235, N297 and P331. In a more specific embodiment the further amino acid substitution is S228P, E233P, L234A, L235A, L235E, N297A, N297D or P331 S. In a particular embodiment the Fc domain comprises amino acid substitutions at positions P329, L234 and L235. In a more particular embodiment the Fc domain comprises the amino acid mutations L234A, L235A and P329G (LALA P329G). This combination of amino acid substitutions almost completely abolishes Fcγ receptor binding of a human IgG Fc domain, as described in European patent application no. EP 11160251.2, incorporated herein by reference in its entirety. EP 11160251.2 also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

In one embodiment the Fc domain is engineered to have decreased effector function, compared to a non-engineered Fc domain. The decreased effector function can include, but is not limited to, one or more of the following: decreased complement dependent cytotoxicity (CDC), decreased antibody-dependent cell-mediated cytotoxicity (ADCC), decreased antibody-dependent cellular phagocytosis (ADCP), decreased cytokine secretion, decreased immune complex-mediated antigen uptake by antigen-presenting

cells, decreased binding to NK cells, decreased binding to macrophages, decreased binding to monocytes, decreased binding to polymorphonuclear cells, decreased direct signaling inducing apoptosis, decreased crosslinking of target-bound antibodies, decreased dendritic cell maturation, or decreased T cell priming.

In one embodiment the decreased effector function is one or more selected from the group of decreased CDC, decreased ADCC, decreased ADCP, and decreased cytokine secretion. In a particular embodiment the decreased effector function is decreased ADCC. In one embodiment the decreased ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or an immunoconjugate comprising a non-engineered Fc domain).

In addition to the Fc domains described hereinabove and in European patent application no. EP 11160251.2, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

IgG<sub>4</sub> antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG<sub>1</sub> antibodies. Hence, in some embodiments the Fc domain of the T cell activating bispecific antigen binding molecules of the invention is an IgG<sub>4</sub> Fc domain, particularly a human IgG<sub>4</sub> Fc domain. In one embodiment the IgG<sub>4</sub> Fc domain comprises amino acid substitutions at position S228, specifically the amino acid substitution S228P. To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG<sub>4</sub> Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E. In another embodiment, the IgG<sub>4</sub> Fc domain comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G. In a particular embodiment, the IgG<sub>4</sub> Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G. Such IgG<sub>4</sub> Fc domain mutants and their Fcγ receptor binding properties are described in European patent application no. EP 11160251.2, incorporated herein by reference in its entirety.

b) Increased Fc Receptor Binding and/or Effector Function

Conversely, there may be situations where it is desirable to maintain or even enhance Fc receptor binding and/or effector functions of immunoconjugates, for example when the immunoconjugate is targeted to a highly specific tumor antigen. Hence, in certain embodiments the Fc domain of the immunoconjugates of the invention is engineered to have increased binding affinity to an Fc receptor. Increased binding affinity may be an increase in the binding affinity of the Fc domain to the Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an Fcγ receptor.

In one embodiment the Fc receptor is selected from the group of FcγRIIIa, FcγRI and FcγRIIa. In a particular embodiment the Fc receptor is FcγRIIIa.

In one such embodiment the Fc domain is engineered to have an altered oligosaccharide structure compared to a non-engineered Fc domain. In a particular such embodiment the Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, compared to a non-engineered

Fc domain. In a more specific embodiment, at least about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 50%, more particularly at least about 70%, of the N-linked oligosaccharides in the Fc domain of the immunoconjugate are non-fucosylated. The non-fucosylated oligosaccharides may be of the hybrid or complex type. In another specific embodiment the Fc domain comprises an increased proportion of bisected oligosaccharides, compared to a non-engineered Fc domain. In a more specific embodiment, at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 50%, more particularly at least about 70%, of the N-linked oligosaccharides in the Fc domain of the immunoconjugate are bisected. The bisected oligosaccharides may be of the hybrid or complex type. In yet another specific embodiment the Fc domain comprises an increased proportion of bisected, non-fucosylated oligosaccharides, compared to a non-engineered Fc domain. In a more specific embodiment, at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 15%, more particularly at least about 25%, at least about 35% or at least about 50%, of the N-linked oligosaccharides in the Fc domain of the immunoconjugate are bisected, non-fucosylated. The bisected, non-fucosylated oligosaccharides may be of the hybrid or complex type.

The oligosaccharide structures in the immunoconjugate Fc domain can be analysed by methods well known in the art, e.g. by MALDI TOF mass spectrometry as described in Umana et al., *Nat Biotechnol* 17, 176-180 (1999) or Ferrara et al., *Biotechnol Bioeng* 93, 851-861 (2006). The percentage of non-fucosylated oligosaccharides is the amount of oligosaccharides lacking fucose residues, relative to all oligosaccharides attached to Asn 297 (e.g. complex, hybrid and high mannose structures) and identified in an N-glycosidase F treated sample by MALDI TOF MS. Asn 297 refers to the asparagine residue located at about position 297 in the Fc domain (EU numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in immunoglobulins. The percentage of bisected, or bisected non-fucosylated, oligosaccharides is determined analogously.

Modification of the glycosylation in the Fc domain of the immunoconjugate may result from production of the immunoconjugate in a host cell that has been manipulated to express altered levels of one or more polypeptides having glycosyltransferase activity.

In one embodiment the Fc domain of the immunoconjugate is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain, by producing the immunoconjugate in a host cell having altered activity of one or more glycosyltransferase. Glycosyltransferases include for example β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), β(1,4)-galactosyltransferase (GalT), β(1,2)-N-acetylglucosaminyltransferase I (GnTI), β(1,2)-N-acetylglucosaminyltransferase II (GnTII) and α(1,6)-fucosyltransferase. In a specific embodiment the Fc domain of the immunoconjugate is engineered to comprise an increased proportion of non-fucosylated oligosaccha-

rides, as compared to a non-engineered Fc domain, by producing the immunoconjugate in a host cell having increased  $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In an even more specific embodiment the host cell additionally has increased  $\alpha$ -mannosidase II (ManII) activity. The glycoengineering methodology that can be used for glycoengineering immunoconjugates of the present invention has been described in greater detail in Umana et al., *Nat Biotechnol* 17, 176-180 (1999); Ferrara et al., *Biotechn Bioeng* 93, 851-861 (2006); WO 99/54342 (U.S. Pat. No. 6,602,684; EP 1071700); WO 2004/065540 (U.S. Pat. Appl. Publ. No. 2004/0241817; EP 1587921), WO 03/011878 (U.S. Pat. Appl. Publ. No. 2003/0175884), the content of each of which is expressly incorporated herein by reference in its entirety.

Generally, any type of cultured cell line, including the cell lines discussed herein, can be used to generate cell lines for the production of immunoconjugates with altered glycosylation pattern. Particular cell lines include CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, and other mammalian cells. In certain embodiments, the host cells have been manipulated to express increased levels of one or more polypeptides having  $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In certain embodiments the host cells have been further manipulated to express increased levels of one or more polypeptides having  $\alpha$ -mannosidase II (ManII) activity. In a specific embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, said Golgi localization domain is the Golgi localization domain of mannosidase II. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in Ferrara et al., *Biotechn Bioeng* 93, 851-861 (2006) and WO 2004/065540, the entire contents of which are expressly incorporated herein by reference.

The host cells which contain a coding sequence of an immunoconjugate of the invention and/or a coding sequence of a polypeptide having glycosyltransferase activity, and which express the biologically active gene products, may be identified e.g. by DNA-DNA or DNA-RNA hybridization, the presence or absence of "marker" gene functions, assessing the level of transcription as measured by the expression of the respective mRNA transcripts in the host cell, or detection of the gene product as measured by immunoassay or by its biological activity—methods which are well known in the art. GnTIII or Man II activity can be detected e.g. by employing a lectin which binds to biosynthesis products of GnTIII or ManII, respectively. An example for such a lectin is the E<sub>4</sub>-PHA lectin which binds preferentially to oligosaccharides containing bisecting GlcNAc. Biosynthesis products (i.e. specific oligosaccharide structures) of polypeptides having GnTIII or ManII activity can also be detected by mass spectrometric analysis of oligosaccharides released from glycoproteins produced by cells expressing said polypeptides. Alternatively, a functional assay which measures the increased effector function and/or increased Fc receptor binding, mediated by immunoconjugates produced by the cells engineered with the polypeptide having GnTIII or ManII activity may be used.

In another embodiment the Fc domain is engineered to comprise an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain, by producing the immunoconjugate in a host cell having decreased  $\alpha(1,6)$ -fucosyltransferase activity. A host cell hav-

ing decreased  $\alpha(1,6)$ -fucosyltransferase activity may be a cell in which the  $\alpha(1,6)$ -fucosyltransferase gene has been disrupted or otherwise deactivated, e.g. knocked out (see Yamane-Ohnuki et al., *Biotech Bioeng* 87, 614 (2004); Kanda et al., *Biotechnol Bioeng* 94(4), 680-688 (2006); Niwa et al., *J Immunol Methods* 306, 151-160 (2006)).

Other examples of cell lines capable of producing defucosylated immunoconjugates include Lec13 CHO cells deficient in protein fucosylation (Ripka et al., *Arch Biochem Biophys* 249, 533-545 (1986); US Pat. Appl. No. US 2003/0157108; and WO 2004/056312, especially at Example 11). The immunoconjugates of the present invention can alternatively be glycoengineered to have reduced fucose residues in the Fc domain according to the techniques disclosed in EP 1 176 195 A1, WO 03/084570, WO 03/085119 and U.S. Pat. Appl. Pub. Nos. 2003/0115614, 2004/093621, 2004/110282, 2004/110704, 2004/132140, U.S. Pat. No. 6,946,292 (Kyowa), e.g. by reducing or abolishing the activity of a GDP-fucose transporter protein in the host cells used for immunoconjugate production.

Glycoengineered immunoconjugates of the invention may also be produced in expression systems that produce modified glycoproteins, such as those taught in WO 2003/056914 (GlycoFi, Inc.) or in WO 2004/057002 and WO 2004/024927 (Greenovation).

In one embodiment the Fc domain of the immunoconjugate is engineered to have increased effector function, compared to a non-engineered Fc domain. The increased effector function can include, but is not limited to, one or more of the following: increased complement dependent cytotoxicity (CDC), increased antibody-dependent cell-mediated cytotoxicity (ADCC), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming.

In one embodiment the increased effector function is one or more selected from the group of increased CDC, increased ADCC, increased ADCP, and increased cytokine secretion. In a particular embodiment the increased effector function is increased ADCC. In one embodiment ADCC induced by an engineered Fc domain (or an immunoconjugate comprising an engineered Fc domain) is a least 2-fold increased as compared to ADCC induced by a non-engineered Fc domain (or an immunoconjugate comprising a non-engineered Fc domain).

#### Effector Moieties

The effector moieties for use in the invention are generally polypeptides that influence cellular activity, for example, through signal transduction pathways. Accordingly, the effector moiety of the immunoconjugate useful in the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response within the cell. For example, an effector moiety of the immunoconjugate can be a cytokine. In particular embodiments the effector moiety is human.

In certain embodiments the effector moiety is a single chain effector moiety. In a particular embodiment the effector moiety is a cytokine. Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-21, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , MIP-1 $\alpha$ , MIP-1 $\beta$ , TGF- $\beta$ , TNF- $\alpha$ , and TNF- $\beta$ . In one embodiment the effector moiety of the

immunoconjugate is a cytokine selected from the group of GM-CSF, IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, IFN- $\alpha$ , IFN- $\gamma$ , MIP-1 $\alpha$ , MIP-1 $\beta$  and TGF- $\beta$ . In one embodiment the effector moiety of the immunoconjugate is a cytokine selected from the group of IL-2, IL-7, IL-10, IL-12, IL-15, IFN- $\alpha$ , and IFN- $\gamma$ . In certain embodiments the cytokine effector moiety is mutated to remove N- and/or O-glycosylation sites. Elimination of glycosylation increases homogeneity of the product obtainable in recombinant production.

In a particular embodiment the effector moiety of the immunoconjugate is IL-2. In a specific embodiment, the IL-2 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity. In another particular embodiment the IL-2 effector moiety is a mutant IL-2 effector moiety having reduced binding affinity to the  $\alpha$ -subunit of the IL-2 receptor. Together with the  $\beta$ - and  $\gamma$ -subunits (also known as CD122 and CD132, respectively), the  $\alpha$ -subunit (also known as CD25) forms the heterotrimeric high-affinity IL-2 receptor, while the dimeric receptor consisting only of the  $\beta$ - and  $\gamma$ -subunits is termed the intermediate-affinity IL-2 receptor. As described in PCT patent application number PCT/EP2012/051991, which is incorporated herein by reference in its entirety, a mutant IL-2 polypeptide with reduced binding to the  $\alpha$ -subunit of the IL-2 receptor has a reduced ability to induce IL-2 signaling in regulatory T cells, induces less activation-induced cell death (AICD) in T cells, and has a reduced toxicity profile in vivo, compared to a wild-type IL-2 polypeptide. The use of such an effector moiety with reduced toxicity is particularly advantageous in an immunoconjugate according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment, the mutant IL-2 effector moiety of the immunoconjugate according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the  $\alpha$ -subunit of the IL-2 receptor (CD25) but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor (consisting of the  $\beta$ - and  $\gamma$ -subunits of the IL-2 receptor), compared to the non-mutated IL-2 effector moiety. In one embodiment the one or more amino acid mutations are amino acid substitutions. In a specific embodiment, the mutant IL-2 effector moiety comprises one, two or three amino acid substitutions at one, two or three position(s) selected from the positions corresponding to residue 42, 45, and 72 of human IL-2. In a more specific embodiment, the mutant IL-2 effector moiety comprises three amino acid substitutions at the positions corresponding to residue 42, 45 and 72 of human IL-2. In an even more specific embodiment, the mutant IL-2 effector moiety is human IL-2 comprising the amino acid substitutions F42A, Y45A and L72G. In one embodiment the mutant IL-2 effector moiety additionally comprises an amino acid mutation at a position corresponding to position 3 of human IL-2, which eliminates the O-glycosylation site of IL-2. Particularly, said additional amino acid mutation is an amino acid substitution replacing a threonine residue by an alanine residue. A particular mutant IL-2 effector moiety useful in the invention comprises four amino acid substitutions at positions corresponding to residues 3, 42, 45 and 72 of human IL-2. Specific

amino acid substitutions are T3A, F42A, Y45A and L72G. As demonstrated in PCT patent application number PCT/EP2012/051991 and in the appended Examples, said quadruple mutant IL-2 polypeptide (IL-2 qm) exhibits no detectable binding to CD25, reduced ability to induce apoptosis in T cells, reduced ability to induce IL-2 signaling in  $T_{reg}$  cells, and a reduced toxicity profile in vivo. However, it retains ability to activate IL-2 signaling in effector cells, to induce proliferation of effector cells, and to generate IFN- $\gamma$  as a secondary cytokine by NK cells.

The IL-2 or mutant IL-2 effector moiety according to any of the above embodiments may comprise additional mutations that provide further advantages such as increased expression or stability. For example, the cysteine at position 125 may be replaced with a neutral amino acid such as alanine, to avoid the formation of disulfide-bridged IL-2 dimers. Thus, in certain embodiments the IL-2 or mutant IL-2 effector moiety of the immunoconjugate according to the invention comprises an additional amino acid mutation at a position corresponding to residue 125 of human IL-2. In one embodiment said additional amino acid mutation is the amino acid substitution C125A.

In a specific embodiment the IL-2 effector moiety of the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 2. In another specific embodiment the IL-2 effector moiety of the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 3.

In another embodiment the effector moiety of the immunoconjugate is IL-12. In a specific embodiment said IL-12 effector moiety is a single chain IL-12 effector moiety. In an even more specific embodiment the single chain IL-12 effector moiety comprises the polypeptide sequence of SEQ ID NO: 4. In one embodiment, the IL-12 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in a NK cell, differentiation in a NK cell, proliferation in a T cell, and differentiation in a T cell.

In another embodiment the effector moiety of the immunoconjugate is IL-10. In a specific embodiment said IL-10 effector moiety is a single chain IL-10 effector moiety. In an even more specific embodiment the single chain IL-10 effector moiety comprises the polypeptide sequence of SEQ ID NO: 5. In another specific embodiment the IL-10 effector moiety is a monomeric IL-10 effector moiety. In a more specific embodiment the monomeric IL-10 effector moiety comprises the polypeptide sequence of SEQ ID NO: 6. In one embodiment, the IL-10 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: inhibition of cytokine secretion, inhibition of antigen presentation by antigen presenting cells, reduction of oxygen radical release, and inhibition of T cell proliferation. An immunoconjugate according to the invention wherein the effector moiety is IL-10 is particularly useful for downregulation of inflammation, e.g. in the treatment of an inflammatory disorder.

In another embodiment the effector moiety of the immunoconjugate is IL-15. In a specific embodiment said IL-15 effector moiety is a mutant IL-15 effector moiety having reduced binding affinity to the  $\alpha$ -subunit of the IL-15 receptor. Without wishing to be bound by theory, a mutant IL-15 polypeptide with reduced binding to the  $\alpha$ -subunit of the IL-15 receptor has a reduced ability to bind to fibroblasts throughout the body, resulting in improved pharmacokinetics and toxicity profile, compared to a wild-type IL-15 polypeptide. The use of an effector moiety with reduced toxicity, such as the described mutant IL-2 and mutant IL-15 effector moieties, is particularly advantageous in an immu-

noconjugate according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment the mutant IL-15 effector moiety of the immunoconjugate according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-15 effector moiety to the  $\alpha$ -subunit of the IL-15 receptor but preserves the affinity of the mutant IL-15 effector moiety to the intermediate-affinity IL-15/IL-2 receptor (consisting of the  $\beta$ - and  $\gamma$ -subunits of the IL-15/IL-2 receptor), compared to the non-mutated IL-15 effector moiety. In one embodiment the amino acid mutation is an amino acid substitution. In a specific embodiment, the mutant IL-15 effector moiety comprises an amino acid substitution at the position corresponding to residue 53 of human IL-15. In a more specific embodiment, the mutant IL-15 effector moiety is human IL-15 comprising the amino acid substitution E53A. In one embodiment the mutant IL-15 effector moiety additionally comprises an amino acid mutation at a position corresponding to position 79 of human IL-15, which eliminates the N-glycosylation site of IL-15. Particularly, said additional amino acid mutation is an amino acid substitution replacing an asparagine residue by an alanine residue. In an even more specific embodiment the IL-15 effector moiety comprises the polypeptide sequence of SEQ ID NO: 7. In one embodiment, the IL-15 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) anti-tumor cytotoxicity.

Mutant cytokine molecules useful as effector moieties in the immunoconjugates can be prepared by deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing. Substitution or insertion may involve natural as well as non-natural amino acid residues. Amino acid modification includes well known methods of chemical modification such as the addition or removal of glycosylation sites or carbohydrate attachments, and the like.

In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is GM-CSF. In a specific embodiment, the GM-CSF effector moiety can elicit proliferation and/or differentiation in a granulocyte, a monocyte or a dendritic cell. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IFN- $\alpha$ . In a specific embodiment, the IFN- $\alpha$  effector moiety can elicit one or more of the cellular responses selected from the group consisting of: inhibiting viral replication in a virus-infected cell, and upregulating the expression of major histocompatibility complex I (MHC I). In another specific embodiment, the IFN- $\alpha$  effector moiety can inhibit proliferation in a tumor cell. In one embodiment the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IFN- $\gamma$ . In a specific embodiment, the IFN- $\gamma$  effector moiety can elicit one or more of the cellular responses selected from the group of: increased macrophage activity, increased expression of MHC molecules, and increased NK cell activity. In one embodiment the effector moiety, particularly a single-chain effector moiety, of the immunocon-

jugate is IL-7. In a specific embodiment, the IL-7 effector moiety can elicit proliferation of T and/or B lymphocytes. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IL-8. In a specific embodiment, the IL-8 effector moiety can elicit chemotaxis in neutrophils. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate, is MIP-1 $\alpha$ . In a specific embodiment, the MIP-1 $\alpha$  effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is MIP-1 $\beta$ . In a specific embodiment, the MIP-1 $\beta$  effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is TGF- $\beta$ . In a specific embodiment, the TGF- $\beta$  effector moiety can elicit one or more of the cellular responses selected from the group consisting of: chemotaxis in monocytes, chemotaxis in macrophages, upregulation of IL-1 expression in activated macrophages, and upregulation of IgA expression in activated B cells.

In one embodiment, the immunoconjugate of the invention binds to an effector moiety receptor with a dissociation constant ( $K_D$ ) that is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 times greater than that for a control effector moiety. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a  $K_D$  that is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 times greater than that for a corresponding immunoconjugate molecule comprising two or more effector moieties. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a dissociation constant  $K_D$  that is about 10 times greater than that for a corresponding immunoconjugate molecule comprising two or more effector moieties.

#### Antigen Binding Moieties

The immunoconjugates of the invention comprise at least one antigen binding moiety. In particular embodiments, the immunoconjugates comprises two antigen binding moieties, i.e. a first and a second antigen binding moiety. In one embodiment the immunoconjugate comprises not more than two antigen binding moieties.

The antigen binding moiety of the immunoconjugate of the invention is generally a polypeptide molecule that binds to a specific antigenic determinant and is able to direct the entity to which it is attached (e.g. an effector moiety and an Fc domain) to a target site, for example to a specific type of tumor cell or tumor stroma that bears the antigenic determinant. The immunoconjugate can bind to antigenic determinants found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, free in blood serum, and/or in the extracellular matrix (ECM).

In certain embodiments the antigen binding moiety is directed to an antigen associated with a pathological condition, such as an antigen presented on a tumor cell or in a tumor cell environment, at a site of inflammation, or on a virus-infected cell.

Non-limiting examples of tumor antigens include MAGE, MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DP-PIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), MAGE-family of tumor antigens (e.g.,

MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-05), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1,  $\alpha$ -fetoprotein, E-cadherin,  $\alpha$ -catenin,  $\beta$ -catenin and  $\gamma$ -catenin, p120ctn, gp100 Pmel117, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotypic, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, lmp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2.

Non-limiting examples of viral antigens include influenza virus hemagglutinin, Epstein-Barr virus LMP-1, hepatitis C virus E2 glycoprotein, HIV gp160, and HIV gp120.

Non-limiting examples of ECM antigens include syndecan, heparanase, integrins, osteopontin, link, cadherins, laminin, laminin type EGF, lectin, fibronectin, notch, tenascin, and matrixin.

The immunoconjugates of the invention can bind to the following specific non-limiting examples of cell surface antigens: FAP, Her2, EGFR, IGF-1R, CD22 (B-cell receptor), CD23 (low affinity IgE receptor), CD30 (cytokine receptor), CD33 (myeloid cell surface antigen), CD40 (tumor necrosis factor receptor), IL-6R (IL6 receptor), CD<sub>20</sub>, MCSP, and PDGF $\beta$ R ( $\beta$  platelet-derived growth factor receptor). In particular embodiments the antigen is a human antigen.

In certain embodiments the antigen-binding moiety is directed to an antigen presented on a tumor cell or in a tumor cell environment. In other embodiments the antigen binding moiety is directed to an antigen presented at a site of inflammation. In a specific embodiment the antigen-binding moiety is directed to an antigen selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcino-embryonic Antigen (CEA), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

In one embodiment, the immunoconjugate of the invention comprises two or more antigen binding moieties, wherein each of these antigen binding moieties specifically binds to the same antigenic determinant.

The antigen binding moiety can be any type of antibody or fragment thereof that retains specific binding to an antigenic determinant. Antibody fragments include, but are not limited to,  $V_H$  fragments,  $V_L$  fragments, Fab fragments,  $F(ab')_2$  fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies (see e.g. Hudson and Souriau, *Nature Med* 9, 129-134 (2003)). In a particular embodiment the antigen binding moiety is a Fab molecule. In one embodiment said Fab molecule is human. In another embodiment said Fab molecule is humanized. In yet another embodiment said Fab molecule comprises human heavy and light chain constant regions.

In one embodiment the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Extra Domain B of fibronectin (EDB). In another embodiment the immunoconjugate comprises at least one, typically two or more antigen binding

moieties that can compete with monoclonal antibody L19 for binding to an epitope of EDB. See, e.g., PCT publication WO 2007/128563 A1 (incorporated herein by reference in its entirety).

In yet another embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 215 or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 213 or a variant thereof that retains functionality. In another embodiment the immunoconjugate comprises a Fab light chain derived from the L19 monoclonal antibody. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 217 or a variant thereof that retains functionality. In yet another embodiment the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 213, SEQ ID NO: 215 and SEQ ID NO: 217, or variants thereof that retain functionality. In another specific embodiment the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 216. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 216. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 214. In yet another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 214. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 218. In yet another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 218.

In one embodiment the immunoconjugate of the invention comprises at least one, typically two or more antigen binding moieties that are specific for the A1 domain of Tenascin C (TNC-A1). In another embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that can compete with monoclonal antibody F16 for binding to an epitope of TNC-A1. See, e.g., PCT publication WO 2007/128563 A1 (incorporated herein by reference in its entirety). In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A1 and/or the A4 domain of Tenascin C (TNC-A1 or TNC-A4 or TNC-A1/A4).

In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable

region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 33 or SEQ ID NO: 35, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 29 or SEQ ID NO: 31, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 33 or SEQ ID NO: 35 or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 29 or SEQ ID NO: 31 or variants thereof that retain functionality. In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 34 or SEQ ID NO: 36. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 34 or SEQ ID NO: 36. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 30 or SEQ ID NO: 32. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 30 or SEQ ID NO: 32.

In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A1 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A1 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment the immunoconjugate comprises both of these polypeptide sequences. In another embodiment, the immunoconjugate further comprises a Fab light chain specific for the A1 domain of Tenascin C. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a particular embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A2 domain of Tenascin C (TNC-A2). In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 27, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, SEQ ID NO: 175, SEQ ID NO: 179, SEQ ID NO: 183 and SEQ ID NO: 187, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light

chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 23, SEQ ID NO: 25; SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 181 and SEQ ID NO: 185, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 27, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, SEQ ID NO: 175, SEQ ID NO: 179, SEQ ID NO: 183 and SEQ ID NO: 187, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 23, SEQ ID NO: 25; SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 181 and SEQ ID NO: 185, or variants thereof that retain functionality. In a particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 27 and the light chain variable region sequence of SEQ ID NO: 25.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 28, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, SEQ ID NO: 176, SEQ ID NO: 180, SEQ ID NO: 184 and SEQ ID NO: 188. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 28, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, SEQ ID NO: 176, SEQ ID NO: 180, SEQ ID NO: 184 and SEQ ID NO: 188. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 178, SEQ ID NO: 182 and SEQ ID NO: 186. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 178, SEQ ID NO: 182 and SEQ ID NO: 186.

In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification, which in turn shares a carboxy-terminal peptide bond with an IL-10 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 235 or SEQ ID NO: 237, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modi-

fication. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 233 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for the A2 domain of Tenascin C. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 239 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 233, SEQ ID NO: 237 and SEQ ID NO: 239 or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 236 or SEQ ID NO: 238. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 236 or SEQ ID NO: 238. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 234. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 234. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 240. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 240.

In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 285, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 287, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for the A2 domain of Tenascin C. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 239 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 285, SEQ ID NO: 287 and SEQ ID NO: 239 or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 286. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 286. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 288. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 288. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 240. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 240.

In a particular embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Fibroblast Activated Protein (FAP). In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 63, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 95, SEQ ID NO: 99, SEQ ID NO: 103, SEQ ID NO: 107, SEQ ID NO: 111, SEQ ID NO: 115, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151 and SEQ ID NO: 155, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 53, SEQ ID NO: 57, SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 105, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149 and SEQ ID NO: 153, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 63, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 95, SEQ ID NO: 99, SEQ ID NO: 103, SEQ ID NO: 107, SEQ ID NO: 111, SEQ ID NO: 115, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151 and SEQ ID NO: 155, or variants thereof that retain

functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 53, SEQ ID NO: 57, SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 105, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149 and SEQ ID NO: 153, or variants thereof that retain functionality. In a particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 111 and the light chain variable region sequence of SEQ ID NO: 109. In a further particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 143 and the light chain variable region sequence of SEQ ID NO: 141. In yet another particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 51 and the light chain variable region sequence of SEQ ID NO: 49.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 64, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 96, SEQ ID NO: 100, SEQ ID NO: 104, SEQ ID NO: 108, SEQ ID NO: 112, SEQ ID NO: 116, SEQ ID NO: 120, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, and SEQ ID NO: 156. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 64, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 96, SEQ ID NO: 100, SEQ ID NO: 104, SEQ ID NO: 108, SEQ ID NO: 112, SEQ ID NO: 116, SEQ ID NO: 120, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, and SEQ ID NO: 156. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 66, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 98, SEQ ID NO: 102, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, and SEQ ID NO: 154. In yet another specific embodiment, the light chain variable

region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 66, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 98, SEQ ID NO: 102, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, and SEQ ID NO: 154.

In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 203, SEQ ID NO: 209, SEQ ID NO: 269, SEQ ID NO: 271 and SEQ ID NO: 273, or variants thereof that retain functionality. In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-15 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 199, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 193, SEQ ID NO: 201 and SEQ ID NO: 207, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for FAP. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205 or SEQ ID NO: 211, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205, the polypeptide sequence of SEQ ID NO: 193, and a polypeptide sequence selected from the group of SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 199 and SEQ ID NO: 269, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 201, SEQ ID NO: 203 and SEQ ID NO: 205, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 207, SEQ ID NO: 209 and SEQ ID NO: 211, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 205, SEQ ID NO: 193 and SEQ ID NO: 269, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 211, SEQ ID NO: 207 and SEQ ID NO: 271, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 211, SEQ ID NO: 207 and SEQ ID NO: 273, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond.

In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification, which in turn shares a carboxy-terminal peptide bond with an IL-10 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 243 or SEQ ID NO: 245, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 241 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for FAP. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 205, SEQ ID NO: 241 and SEQ ID NO: 243, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 205, SEQ ID NO: 241 and SEQ ID NO: 245, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 196, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 204, SEQ ID NO: 210, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 270, SEQ ID NO: 272 and SEQ ID NO: 274. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 196, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 204, SEQ ID NO: 210, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 270, SEQ ID NO: 272 and SEQ ID NO: 274. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 194, SEQ ID NO: 202, SEQ ID NO: 208 and SEQ ID NO: 242. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 194, SEQ ID NO: 202, SEQ ID NO: 208 and SEQ ID NO: 242. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 206 or SEQ ID NO: 212. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 206 or SEQ ID NO: 212.

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Carcinoembryonic Antigen (CEA). In a specific embodiment, the antigen binding moieties of

the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 191 or SEQ ID NO: 295, or a variant thereof that retains functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 189 or SEQ ID NO: 293, or a variant thereof that retains functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 191, or a variant thereof that retains functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 189, or a variant thereof that retains functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 295, or a variant thereof that retains functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 293, or a variant thereof that retains functionality.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 192 or SEQ ID NO: 296. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of SEQ ID NO: 192 or SEQ ID NO: 296. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 190 or SEQ ID NO: 294. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of SEQ ID NO: 190 or SEQ ID NO: 294.

In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group consisting of SEQ ID NO: 229, SEQ ID NO: 275, SEQ ID NO: 277 and SEQ ID NO: 279, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 227 or SEQ ID NO: 281, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for CEA. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 231 or SEQ ID NO: 283, or a variant thereof that retains functionality. In another

embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 227, SEQ ID NO: 229 and SEQ ID NO: 231, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 275, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 277, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 279, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain polypeptide chains comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of SEQ ID NO: 230, SEQ ID NO: 276, SEQ ID NO: 278 and SEQ ID NO: 280. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence selected from the group consisting of SEQ ID NO: 230, SEQ ID NO: 276, SEQ ID NO: 278 and SEQ ID NO: 280. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 228 or SEQ ID NO: 282. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 232 or SEQ ID NO: 284. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 232 or SEQ ID NO: 284.

In some embodiments the immunoconjugate comprises a polypeptide sequence wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 247, SEQ ID NO: 249 and SEQ ID NO: 251, or a variant thereof that retains functionality. In one such embodiment the immunoconjugate further comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate further comprises a polypeptide sequence selected from the group of SEQ ID NO: 193, SEQ ID NO: 201 and SEQ ID NO: 207, or a variant thereof that retains functionality. In another such embodiment the immunoconjugate further comprises a polypeptide sequence wherein a Fab heavy chain specific for EDB, TNC A1, TNC A2 or CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G. According to any of the above embodiments the immunoconjugate may further comprise a Fab light chain specific for the corresponding antigen.

Immunoconjugates of the invention include those that have sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in SEQ ID NOs 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 and 287, including functional fragments or variants thereof. The invention also encompasses immunoconjugates comprising these sequences with conservative amino acid substitutions. Polynucleotides

The invention further provides isolated polynucleotides encoding an immunoconjugate as described herein or a fragment thereof.

Polynucleotides of the invention include those that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in SEQ ID NOs 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286 and 288, including functional fragments or variants thereof.

The polynucleotides encoding immunoconjugates of the invention may be expressed as a single polynucleotide that encodes the entire immunoconjugate or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional immunoconjugate. For example, the light chain portion of an antigen binding moiety may be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of the antigen binding moiety, an Fc domain subunit and optionally the effector moiety. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antigen binding moiety. In another example, the portion of the immunoconjugate comprising the heavy chain portion of a first antigen binding moiety, one of the two Fc domain subunits and the effector moiety could be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of a second antigen binding moiety and the other of the two Fc domain subunits. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In one embodiment, an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and a single effector moiety, wherein the antigen binding moiety is an antigen binding domain comprising a heavy chain variable region and a light chain variable region, particularly a Fab molecule. In one embodiment, an isolated polynucleotide of the invention encodes the heavy chain of the first antigen binding moiety, a subunit of the Fc domain, and the effector moiety. In another embodiment, an isolated polynucleotide of the invention

encodes the heavy chain of the first antigen binding moiety and a subunit of the Fc domain. In yet another embodiment, an isolated polynucleotide of the invention encodes a subunit of the Fc domain and the effector moiety. In a more specific embodiment the isolated polynucleotide encodes a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit. In another specific embodiment the isolated polynucleotide encodes a polypeptide wherein an Fc domain subunit shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In yet another specific embodiment, the isolated polynucleotide encodes a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, which in turn shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In yet another specific embodiment the isolated polynucleotide encodes a polypeptide wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit.

In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence as shown in SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 293 or 295. In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence as shown in SEQ ID NO 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287. In another embodiment, the invention is further directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence shown SEQ ID NO 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 294, 296, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 270, 272, 274, 276, 278, 280, 282, 284, 286 or 288. In another embodiment, the invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a nucleic acid sequence shown in SEQ ID NO 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 294, 296, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 270, 272, 274, 276, 278, 280, 282, 284, 286 or 288. In another embodiment, the invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a

sequence that encodes a variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 293 or 295. In another embodiment, the invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287. The invention encompasses an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes the variable region sequences of SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 293 or 295 with conservative amino acid substitutions. The invention also encompasses an isolated polynucleotide encoding an immunoconjugate of the invention or fragment thereof, wherein the polynucleotide comprises a sequence that encodes the polypeptide sequences of SEQ ID NO 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287 with conservative amino acid substitutions.

In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

#### Untargeted Conjugates

The invention provides not only immunoconjugates targeted to a specific antigen (e.g. a tumor antigen) but also untargeted conjugates comprising one or more Fab molecules which do not specifically bind to any antigen, particularly not bind to any human antigen. The absence of specific binding of these conjugates to any antigen (i.e. the absence of any binding that can be discriminated from non-specific interaction) can be measured e.g. by ELISA or surface plasmon resonance as described herein. Such conjugates are particularly useful e.g. for enhancing the serum half life of the effector moiety they comprise, as compared to the serum half-life of the unconjugated effector moiety, where targeting to a particular tissue is not desired.

Specifically, the invention provides a conjugate comprising a first Fab molecule which does not specifically bind any antigen, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. More specifically, the invention provides a conjugate comprising a first Fab molecule comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. Like the immunoconjugates of the invention, the con-

jugates can have a variety of configurations, as described above under “Immunoconjugate Formats” (the antigen binding moiety of the immunoconjugate being replaced by a Fab molecule which does not specifically bind to any antigen, such as a Fab molecule comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297). Likewise, the features of the Fc domain as well as the effector moiety as described above under “Fc domain” and “Effector moieties” for the immunoconjugates of the invention equally apply, alone or in combination, to the untargeted conjugates of the invention.

In a particular embodiment, the conjugate comprises (i) an immunoglobulin molecule, comprising a first and a second Fab molecule which do not specifically bind any antigen and an Fc domain, and (ii) an effector moiety, wherein not more than one effector moiety is present and wherein the immunoglobulin molecule is a human IgG1 subclass immunoglobulin; the Fc domain comprises a knob modification in one and a hole modification in the other one of its two subunits, and the amino acid substitutions L234A, L235A and P329G in each of its subunits; and the effector moiety is an IL-2 molecule fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide. In a specific embodiment, the conjugate comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297.

In certain embodiments, the conjugate comprises (i) an immunoglobulin molecule, comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297, and (ii) an effector moiety, wherein not more than one effector moiety is present. In one such embodiment the immunoglobulin molecule is a human IgG1 subclass immunoglobulin. In one such embodiment the Fc domain comprises a knob modification in one and a hole modification in the other one of its two subunits. In a specific such embodiment, the Fc domain comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits. In yet another such embodiment, the effector moiety is an IL-2 molecule fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In one embodiment the conjugate comprises a polypeptide sequence wherein a Fab heavy chain which does not specifically bind to any antigen shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the conjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 289 and SEQ ID NO: 291, or a variant thereof that retains functionality. In one embodiment the conjugate comprises a polypeptide sequence wherein a Fab heavy chain which does not specifically bind to any antigen shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the conjugate comprises the polypeptide sequence of SEQ ID NO: 219, or a variant thereof that retains functionality. In another embodiment, the conjugate comprises a Fab light chain which does not specifically bind any antigen. In a more specific embodiment, the conjugate comprises the polypeptide sequence of SEQ ID NO: 225, or a variant thereof that retains functionality. In another embodiment, the conjugate comprises the polypeptide

another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 223 and SEQ ID NO: 225, or variants thereof that retain functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 289 and SEQ ID NO: 225, or variants thereof that retain functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 291 and SEQ ID NO: 225, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain polypeptide chains comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the conjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence selected from the group consisting of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292.

The invention also provides an isolated polynucleotide encoding the conjugate of the invention of a fragment thereof. In a specific embodiment, the isolated polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the a sequence selected from the group of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292. The invention further provides an expression vector comprising the isolated polynucleotide, and a host cell comprising the isolated polynucleotide or the expression vector of the invention. In another aspect is provided a method of producing the conjugate of the invention, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the conjugate and b) recovering the conjugate. The invention also encompasses a conjugate produced by the method of the invention. The disclosure provided herein in relating to methods of producing the immunoconjugates of the invention (see e.g. under “Recombinant Methods”) can equally be applied to the conjugates of the invention.

The invention further provides a pharmaceutical composition comprising the conjugate of the invention and a pharmaceutically acceptable carrier. The disclosure provided herein in relating to pharmaceutical compositions of the immunoconjugates of the invention (see e.g. under “Compositions, Formulations, and Routes of Administration”) can equally be applied to the conjugates of the invention. Furthermore, the conjugate can be employed in the methods of use described herein for the immunoconjugates of the invention. The disclosure provided herein in relating to methods of using the immunoconjugates of the invention in the treatment of disease (see e.g. under “Therapeutic Methods and Compositions”, “Other Agents and Treatments” and “Articles of manufacture”) can equally be applied to the conjugates of the invention.

Recombinant Methods

Immunoconjugates of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For

recombinant production one or more polynucleotide encoding the immunoconjugate (fragment), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an immunoconjugate (fragment) along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the immunoconjugate (fragment) (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the immunoconjugate (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription

termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit d-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the immunoconjugate is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding an immunoconjugates of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, e.g. an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse  $\beta$ -glucuronidase. Exemplary amino acid and corresponding polynucleotide sequences of secretory signal peptides are shown in SEQ ID NOs 8-16.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the immunoconjugate may be included within or at the ends of the immunoconjugate (fragment) encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combina-

tion, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) an immunoconjugate of the invention. As used herein, the term “host cell” refers to any kind of cellular system which can be engineered to generate the immunoconjugates of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of immunoconjugates are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the immunoconjugate for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized”, resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gemgross, Nat Biotech 22, 1409-1414 (2004), and Li et al., Nat Biotech 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., J Gen Virol 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol Reprod 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TR1 cells (as described, e.g., in Mather et al., Annals N.Y. Acad Sci 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr<sup>-</sup> CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, N.J.), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell,

such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., YO, NS0, Sp20 cell). Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain.

In one embodiment, a method of producing an immunoconjugate according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the immunoconjugate, as provided herein, under conditions suitable for expression of the immunoconjugate, and recovering the immunoconjugate from the host cell (or host cell culture medium).

The components of the immunoconjugate are genetically fused to each other. Immunoconjugates can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between the effector moiety and the Fc domain are found in the sequences shown in SEQ ID NO 195, 197, 199, 203, 209, 215, 229, 235, 237, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279 and 285. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

In certain embodiments the one or more antigen binding moieties of the immunoconjugate comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, “Antibodies, a laboratory manual”, Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. Pat. No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Pat. No. 5,969,108 to McCafferty). Antigen binding moieties and methods for producing the same are also described in detail in PCT publication WO 2011/020783, the entire content of which is incorporated herein by reference.

Any animal species of antibody, antibody fragment, antigen binding domain or variable region can be used in the immunoconjugates of the invention. Non-limiting antibodies, antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the immunoconjugate is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can also be prepared in accordance with methods well known in the art (see e.g. U.S. Pat. No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-deter-

mining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but “cloaking” them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front Biosci* 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332, 323-329 (1988); Queen et al., *Proc Natl Acad Sci USA* 86, 10029-10033 (1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., *Nature* 321, 522-525 (1986); Morrison et al., *Proc Natl Acad Sci* 81, 6851-6855 (1984); Morrison and 01, *Adv Immunol* 44, 65-92 (1988); Verhoeven et al., *Science* 239, 1534-1536 (1988); Padlan, *Molec Immun* 31(3), 169-217 (1994); Kashmiri et al., *Methods* 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol Immunol* 28, 489-498 (1991) (describing “resurfacing”); Dall’Acqua et al., *Methods* 36, 43-60 (2005) (describing “FR shuffling”); and Osbourn et al., *Methods* 36, 61-68 (2005) and Klimka et al., *Br J Cancer* 83, 252-260 (2000) (describing the “guided selection” approach to FR shuffling). Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, *Nat Biotech* 23, 1117-1125 (2005)). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in *Methods in Molecular Biology* 178, 1-37 (O’Brien et al., ed., Human Press, Totowa, N.J., 2001); and McCafferty et al., *Nature* 348, 552-554; Clackson et al., *Nature* 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. A detailed description of the preparation of antigen binding moieties for immunoconjugates by phage display can be found in the Examples appended to PCT publication WO 2011/020783.

In certain embodiments, the antigen binding moieties useful in the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in PCT publication WO 2011/020783 (see Examples relating to affinity maturation) or U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the immunoconjugate of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljebblad, et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). Competition assays may be used to identify an antibody, antibody fragment, antigen binding domain or variable domain that competes with a reference antibody for binding to a particular antigen, e.g. an antibody that competes with the L19 antibody for binding to the Extra Domain

B of fibronectin (EDB). In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) “Epitope Mapping Protocols,” in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, N.J.). In an exemplary competition assay, immobilized antigen (e.g. EDB) is incubated in a solution comprising a first labeled antibody that binds to the antigen (e.g. L19 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to the antigen. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch. 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

Immunoconjugates prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the immunoconjugate binds. For example, for affinity chromatography purification of immunoconjugates of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an immunoconjugate essentially as described in the Examples. The purity of the immunoconjugate can be determined by any of a variety of well known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like. For example, the heavy chain fusion proteins expressed as described in the Examples were shown to be intact and properly assembled as demonstrated by reducing SDS-PAGE (see e.g. FIG. 4). Three bands were resolved at approximately Mr 25,000, Mr 50,000 and Mr 60,000, corresponding to the predicted molecular weights of the immunoglobulin light chain, heavy chain and heavy chain/effector moiety fusion protein.

#### Assays

Immunoconjugates provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

#### Affinity Assays

The affinity of the immunoconjugate for an effector moiety receptor (e.g. IL-10R or various forms of IL-2R), an Fc receptor, or a target antigen, can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of immunoconjugates for different receptors or target antigens may be

evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following and in the Examples below.

According to one embodiment,  $K_D$  is measured by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25° C. with ligand (e.g. effector moiety receptor, Fc receptor or target antigen) immobilized on CM5 chips. Briefly, carboxymethylated dextran biosensor chips (CM5, GE Healthcare) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Recombinant ligand is diluted with 10 mM sodium acetate, pH 5.5, to 0.5-30 µg/ml before injection at a flow rate of 10 µl/minute to achieve approximately 100-5000 response units (RU) of coupled protein. Following the injection of the ligand, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, three- to five-fold serial dilutions of immunoconjugate (range between ~0.01 nM to 300 nM) are injected in HBS-EP+ (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) at 25° C. at a flow rate of approximately 30-50 µl/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) are calculated using a simple one-to-one Langmuir binding model (BIACORE® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_D$ ) is calculated as the ratio  $k_{off}/k_{on}$ . See, e.g., Chen et al., *J Mol Biol* 293, 865-881 (1999).

#### Activity Assays

Biological activity of the immunoconjugates of the invention can be measured by various assays as described in the Examples. Biological activities may for example include the induction of proliferation of effector moiety receptor-bearing cells, the induction of signaling in effector moiety receptor-bearing cells, the induction of cytokine secretion by effector moiety receptor-bearing cells, and the induction of tumor regression and/or the improvement of survival.

#### Compositions, Formulations, and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising any of the immunoconjugates provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises any of the immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises any of the immunoconjugates provided herein and at least one additional therapeutic agent, e.g., as described below.

Further provided is a method of producing an immunoconjugate of the invention in a form suitable for administration in vivo, the method comprising (a) obtaining an immunoconjugate according to the invention, and (b) formulating the immunoconjugate with at least one pharmaceutically acceptable carrier, whereby a preparation of immunoconjugate is formulated for administration in vivo.

Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more immunoconjugate dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one immunoconjugate and

optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g. antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The composition may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. Immunoconjugates of the present invention (and any additional therapeutic agent) can be administered intravenously, intradermally, intraarterially, intraperitoneally, intrasplenicly, intracranially, intraarticularly, intraprostatically, intrasplenicly, intrareally, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation (e.g. aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (e.g. liposomes), or by other method or any combination of the foregoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference). Parenteral administration, in particular intravenous injection, is most commonly used for administering polypeptide molecules such as the immunoconjugates of the invention.

Parenteral compositions include those designed for administration by injection, e.g. subcutaneous, intradermal, intrasplenic, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the immunoconjugates of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the immunoconjugates may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the immunoconjugates of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incor-

porating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyltrimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

In addition to the compositions described previously, the immunoconjugates may also be formulated as a depot prepa-

ration. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the immunoconjugates may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the immunoconjugates of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The immunoconjugates may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms. Therapeutic Methods and Compositions

Any of the immunoconjugates provided herein may be used in therapeutic methods. Immunoconjugates of the invention can be used as immunotherapeutic agents, for example in the treatment of cancers.

For use in therapeutic methods, immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

In one aspect, immunoconjugates of the invention for use as a medicament are provided. In further aspects, immunoconjugates of the invention for use in treating a disease are provided. In certain embodiments, immunoconjugates of the invention for use in a method of treatment are provided. In one embodiment, the invention provides an immunoconjugate as described herein for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides an immunoconjugate for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the immunoconjugate. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if

the disease to be treated is cancer. An "individual" according to any of the above embodiments is a mammal, preferably a human.

In a further aspect, the invention provides for the use of an immunoconjugate of the invention in the manufacture or preparation of a medicament for the treatment of a disease in an individual in need thereof. In one embodiment, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective amount of the medicament. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In one embodiment, the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above embodiments may be a mammal, preferably a human.

In a further aspect, the invention provides a method for treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of an immunoconjugate of the invention. In one embodiment a composition is administered to said individual, comprising immunoconjugate of the invention in a pharmaceutically acceptable form. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above embodiments may be a mammal, preferably a human.

In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer. Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using an immunoconjugate of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer. In some embodiments, particularly where the effector moiety of the immunoconjugate is IL-10, the disease to be treated is an inflammatory disorder. Non-limiting examples of inflammatory disorders include rheumatoid arthritis, psoriasis or Crohn's disease. A skilled artisan readily recognizes that in many cases the immunoconjugates may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of immunoconjugate that provides a physiological change is considered an "effective amount" or a "therapeu-

tically effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

The immunoconjugates of the invention are also useful as diagnostic reagents. The binding of an immunoconjugate to an antigenic determinant can be readily detected by using a secondary antibody specific for the effector moiety. In one embodiment, the secondary antibody and the immunoconjugate facilitate the detection of binding of the immunoconjugate to an antigenic determinant located on a cell or tissue surface.

In some embodiments, an effective amount of an immunoconjugate of the invention is administered to a cell. In other embodiments, a therapeutically effective amount of an immunoconjugates of the invention is administered to an individual for the treatment of disease.

For the prevention or treatment of disease, the appropriate dosage of an immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of immunoconjugate, the severity and course of the disease, whether the immunoconjugate is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the immunoconjugate, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg-10 mg/kg) of immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the immunoconjugate would be in the range from about 0.005 mg/kg to about 10 mg/kg. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg body weight, about 5 microgram/kg body weight, about 10 microgram/kg body weight, about 50 microgram/kg body weight, about 100 microgram/kg body weight, about 200 microgram/kg body weight, about 350 microgram/kg body weight, about 500 microgram/kg body weight, about 1 milligram/kg body weight, about 5 milligram/kg body weight, about 10 milligram/kg body weight, about 50 milligram/kg body weight, about 100 milligram/kg body weight, about 200 milligram/kg body weight, about 350 milligram/kg body weight, about 500 milligram/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 microgram/kg body weight to about 500 milligram/kg body weight, etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be

administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The immunoconjugates of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the immunoconjugates of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the  $IC_{50}$  as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from in vivo data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the immunoconjugates which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

In cases of local administration or selective uptake, the effective local concentration of the immunoconjugates may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the immunoconjugates described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of an immunoconjugate can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the  $LD_{50}$  (the dose lethal to 50% of a population) and the  $ED_{50}$  (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio  $LD_{50}/ED_{50}$ . Immunoconjugates that exhibit large therapeutic indices are preferred. In one embodiment, the immunoconjugate according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the  $ED_{50}$  with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: *The Pharmacological Basis of Therapeutics*, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with immunoconjugates of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

#### Other Agents and Treatments

The immunoconjugates of the invention may be administered in combination with one or more other agents in therapy. For instance, an immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers. In a particular embodiment, the additional therapeutic agent is an anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of immunoconjugate used, the type of disorder or treatment, and other factors discussed above. The immunoconjugates are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Immunoconjugates of the invention can also be used in combination with radiation therapy.

#### Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a

sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

#### EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

##### Example 1

##### General Methods

##### Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook et al., *Molecular cloning: A laboratory manual*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. The molecular biological reagents were used according to the manufacturer's instructions. General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E. A. et al., (1991) *Sequences of Proteins of Immunological Interest*, Fifth Ed., NIH Publication No 91-3242.

##### DNA Sequencing

DNA sequences were determined by double strand sequencing.

##### Gene Synthesis

Desired gene segments where required were either generated by PCR using appropriate templates or were synthesized by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. In cases where no exact gene sequence was available, oligonucleotide primers were designed based on sequences from closest homologues and the genes were isolated by RT-PCR from RNA originating from the appropriate tissue. The gene segments flanked by singular restriction endonuclease cleavage sites were cloned into standard cloning/sequencing vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding

for a leader peptide which targets proteins for secretion in eukaryotic cells. SEQ ID NOs 8-16 give exemplary leader peptides and polynucleotide sequences encoding them.

##### Preparation of IL-2R $\beta\gamma$ Subunit-Fc Fusions and IL-2R $\alpha$ Subunit Fc Fusion

To study IL-2 receptor binding affinity, a tool was generated that allowed for the expression of a heterodimeric IL-2 receptor; the  $\beta$ -subunit of the IL-2 receptor was fused to an Fc molecule that was engineered to heterodimerize (Fc(hole)) (see SEQ ID NOs 17 and 18) using the "knobs-into-holes" technology (Merchant et al., *Nat. Biotech.* 16, 677-681 (1998)). The  $\gamma$ -subunit of the IL-2 receptor was then fused to the Fc(knob) variant (see SEQ ID NOs 19 and 20), which heterodimerized with Fc(hole). This heterodimeric Fc-fusion protein was then used as a substrate for analyzing the IL-2/IL-2 receptor interaction. The IL-2R  $\alpha$ -subunit was expressed as monomeric chain with an AcTev cleavage site and an Avi His tag (SEQ ID NOs 21 and 22). The respective IL-2R subunits were transiently expressed in HEK EBNA 293 with serum for the IL-2R  $\gamma\gamma$  subunit construct and without serum for the  $\alpha$ -subunit construct. The IL-2R  $\beta\gamma$  subunit construct was purified on protein A (GE Healthcare), followed by size exclusion chromatography (GE Healthcare, Superdex 200). The IL-2R  $\alpha$ -subunit was purified via His tag on a NiNTA column (Qiagen) followed by size exclusion chromatography (GE Healthcare, Superdex 75). Amino acid and corresponding nucleotide sequences of various receptor constructs are given in SEQ ID NOs 17-22 and 255-268.

##### Preparation of Immunconjugates

Details about the generation and affinity maturation of antigen binding moieties directed to FAP can be found in the Examples appended to PCT patent application publication no. WO 2012/020006, which is incorporated herein by reference in its entirety. As described therein, various antigen binding domains directed to FAP have been generated by phage display, including the ones designated 4G8, 28H1 and 4B9 used in the following examples. Clone 28H1 is an affinity matured antibody based on parental clone 4G8, while clone 4B9 is an affinity matured antibody based on parental clone 3F2. The antigen binding domain designated 2B10 used herein is directed to the A2 domain of Tenascin C (TNC A2). Details about this and other antigen binding moieties directed against TNC A2 can be found in PCT patent application publication no. WO 2012/020038, which is incorporated herein by reference in its entirety. The antigen binding domain designated L19, directed against the Extra Domain B (EDB) of fibronectin is derived from the L19 antibody described in PCT publication WO 2007/128563. The antigen binding domains designated CH1A1A and CH1A1A 98/99 2F1 used herein are directed to CEA, and are described in more detail in PCT patent application no. PCT/EP2012/053390, which is incorporated herein by reference in its entirety.

The IL-2 quadruple mutant (qm) used as effector moiety in some of the following examples is described in detail in PCT patent application no. PCT/EP2012/051991, which is incorporated herein by reference in its entirety. Briefly, IL-2 qm is characterized by the following mutations:

1. T3A—knockout of predicted O-glycosylation site
2. F42A—knockout of IL-2/IL-2R  $\alpha$  interaction
3. Y45A—knockout of IL-2/IL-2R  $\alpha$  interaction
4. L72G—knockout of IL-2/IL-2R  $\alpha$  interaction
5. C125A—mutation to avoid disulfide-bridged IL-2 dimers

The T3A mutation was chosen to eliminate the O-glycosylation site and obtain a protein product with higher homogeneity and purity when the IL-2 qm polypeptide or an

immunoconjugate comprising it is expressed in eukaryotic cells such as CHO or HEK293 cells. The three mutations F42A, Y45A and L72G were chosen to interfere with the binding to CD25, the  $\alpha$ -subunit of the IL-2 receptor. Reduced or abolished CD25 binding results in reduced activation-induced cell death (AICD), lack of preferential activation of regulatory T cells, as well as reduced toxicity (as described in EP 11153964.9).

The DNA sequences were generated by gene synthesis and/or classical molecular biology techniques and subcloned into mammalian expression vectors under the control of an MPSV promoter and upstream of a synthetic polyA site, each vector carrying an EBV OriP sequence. Immunoconjugates as applied in the examples below were produced by co-transfecting exponentially growing HEK293-EBNA cells with the mammalian expression vectors using calcium phosphate-transfection. Alternatively, HEK293 cells growing in suspension were transfected by polyethylenimine (PEI) with the respective expression vectors. Alternatively, stably transfected CHO cell pools or CHO cell clones were used for production in serum-free media. Subsequently, the IgG-cytokine fusion proteins were purified from the supernatant. Briefly, IgG-cytokine fusion proteins were purified by one affinity step with protein A (HiTrap ProtA, GE Healthcare) equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. After loading of the supernatant, the column was first washed with 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5 and subsequently washed with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. The IgG-cytokine fusion protein was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3. Fractions were neutralized and pooled and purified by size exclusion chromatography (HiLoad 16/60 Superdex 200, GE Healthcare) in final formulation buffer: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7. Exemplary detailed purification procedures and results are given for selected constructs below. The protein concentration of purified protein samples was determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of immunoconjugates were analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiothreitol) and stained with Coomassie blue (SimpleBlue™ SafeStain, Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instructions (4-20% Tris-glycine gels or 3-12% Bis-Tris). The aggregate content of immunoconjugate samples was analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare) in 2 mM MOPS, 150 mM NaCl, 0.02% NaN<sub>3</sub>, pH 7.3 running buffer at 25° C. The integrity of the amino acid backbone of reduced antibody light and heavy chains can be verified by NanoElectrospray Q-TOF mass spectrometry after removal of N-glycans by enzymatic treatment with Peptide-N Glycosidase F (Roche Molecular Biochemicals). The oligosaccharides attached to the Fc domain of the immunoconjugates are analysed by MALDI TOF-MS as described below. Oligosaccharides are enzymatically released from the immunoconjugates by PNGaseF digestion. The resulting digest solution containing the released oligosaccharides is either prepared directly for MALDI TOF-MS analysis or is further digested with EndoH glycosidase prior to sample preparation for MALDI TOF-MS analysis.

#### Example 2

FAP-targeted IgG-IL-2 qm fusion proteins were generated based on the FAP-antibodies 4G8, 28H1 and 4B9, wherein

one single IL-2 quadruple mutant (qm) was fused to the C-terminus of one heterodimeric heavy chain as shown in FIG. 2A. Targeting to the tumor stroma where FAP is selectively expressed is achieved via the bivalent antibody Fab region (avidity effect). Heterodimerization resulting in the presence of a single IL-2 quadruple mutant is achieved by application of the knob-into-hole technology. In order to minimize the generation of homodimeric IgG-cytokine fusions the cytokine was fused to the C-terminus (with deletion of the C-terminal Lys residue) of the knob-containing IgG heavy chain via a (G<sub>4</sub>S)<sub>3</sub> or G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker. The antibody-cytokine fusion has IgG-like properties. To reduce FcγR binding/effector function and prevent FcR co-activation, P329G L234A L235A (LALA) mutations were introduced in the Fc domain. The sequences of these immunoconjugates are given SEQ ID NOs 193, 269 and 205 (28H1 with (G<sub>4</sub>S)<sub>3</sub> linker), SEQ ID NOs 193, 195 and 205 (28H1 with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 201, 203 and 205 (4G8 with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 207, 209 and 211 (4B9 with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 207, 271 and 211 (4B9 with (G<sub>4</sub>S)<sub>3</sub> linker).

In addition, a CEA-targeted IgG-IL-2 qm fusion protein based on the anti-CEA antibody CH1A1A 98/99 2F1, a control DP47GS non-targeted IgG-IL-2 qm fusion protein wherein the IgG does not bind to a specified target, as well as a tumor stroma specific 2B10-based IgG-IL-2 qm fusion protein targeted against the A2 domain of tenascin-C were generated. The sequences of these immunoconjugates are given in SEQ ID NOs 275, 281 and 283 (CH1A1A 98/99 2F1 with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 277, 281 and 283 (CH1A1A 98/99 2F1 with (G<sub>4</sub>S)<sub>3</sub> linker), SEQ ID NOs 219, 221 and 225 (DP47GS with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 219, 289 and 225 (DP47GS with (G<sub>4</sub>S)<sub>3</sub> linker), SEQ ID NOs 285, 287 and 239 (2B10 with (G<sub>4</sub>S)<sub>3</sub> linker). The constructs were generated by transient expression in HEK293 EBNA cells and purified as described above. FIGS. 3 to 9 show exemplary chromatograms and elution profiles of the purification (A, B) as well as the analytical SDS-PAGE and size exclusion chromatographies of the final purified constructs (C, D). Transient expression yields were 42 mg/L for the 4G8-based, 20 mg/L for the 28H1-based, 10 mg/L for the 4B9-based, 5.3 mg/L for the CH1A1A 98/99 2F1-based, 36.7 mg/L for the 2B10-based and 13.8 mg/L for the DP47GS-based IgG-IL-2 qm immunoconjugate.

In addition a 28H1-based FAP-targeted IgG-IL-15 immunoconjugate is being generated, the sequences of which are given in SEQ ID NOs 193, 199 and 205. In the IL-15 polypeptide sequence the glutamic acid residue at position 53 is replaced by alanine to reduce binding to the  $\alpha$ -subunit of the IL-15 receptor, and the asparagine residue at position 79 is replaced by alanine to abolish glycosylation. The IgG-IL-15 fusion protein is generated by transient expression and purified as described above.

#### FAP Binding Affinity

The FAP binding activity of the IgG-IL-2 qm immunoconjugates based on 4G8 and 28H1 anti-FAP antibodies were determined by surface plasmon resonance (SPR) on a Biacore machine in comparison to the corresponding unmodified IgG antibodies. Briefly, an anti-His antibody (Penta-His, Qiagen 34660) was immobilized on CM5 chips to capture 10 nM His-tagged human FAP (20 s). Temperature was 25° C. and HBS-EP was used as buffer. Analyte concentration was 50 nM down to 0.05 nM at a flow rate of 50  $\mu$ l/min (association: 300 s, dissociation: 900 s, regeneration: 60 s with 10 mM glycine pH 2). Fitting was performed based on a 1:1 binding model, RI=0, Rmax=local (because of capture format). The following table gives the estimated

apparent bivalent affinities (pM avidity) as determined by SPR fitted with 1:1 binding  $RI=0$ ,  $R_{max}=local$ .

	Hu FAP
4G8 IgG-IL-2 qm	100 pM
4G8 IgG	50 pM
28H1 IgG-IL-2 qm	175 pM
28H1 IgG	200 pM

The data show that within the error of the method affinity for human FAP is retained for the 28H1-based immunoconjugate or only slightly decreased for the 4G8-based immunoconjugate as compared to the corresponding unmodified antibodies.

Similarly, the affinity ( $K_D$ ) of 4B9 IgG-IL-2 qm (16 pM), CH1A1A 98/99 2F1 IgG-IL-2 qm (400 pM), CH1A1A 98/99 2F1 IgG-IL-2 wt (see Example 4; 470 pM) and 2B10 IgG-IL-2 qm (150 pM, vs. 300 pM for unconjugated 2B10 IgG) to human FAP, CEA and TNC A2, respectively, were determined by SPR at 25° C. Cross-reactivity of the 4B9 and 2B10 antibodies to human, murine and cynomolgus FAP or TNC A2, respectively, was also confirmed.

Subsequently, the affinity of the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates to the IL-2R  $\beta\gamma$  heterodimer and the IL-2R  $\alpha$ -subunit were determined by surface plasmon resonance (SPR) in direct comparison to the Fab-IL-2 qm-Fab immunoconjugate format described in PCT patent application no. PCT/EP2012/051991. Briefly, the ligands—either the human IL-2R  $\alpha$ -subunit or the human IL-2R  $\beta\gamma$  heterodimer—were immobilized on a CM5 chip. Subsequently, the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates or the 4G8- and 28H1-based Fab-IL-2 qm-Fab immunoconjugates for comparison were applied to the chip as analytes at 25° C. in HBS-EP buffer in concentrations ranging from 300 nM down to 1.2 nM (1:3 dil.). Flow rate was 30  $\mu$ l/min and the following conditions were applied for association: 180 s, dissociation: 300 s, and regeneration: 2 $\times$ 30 s with 3 M  $MgCl_2$  for IL-2R  $\beta\gamma$  heterodimer, 10 s with 50 mM NaOH for IL-2R  $\alpha$ -subunit. 1:1 binding was applied for fitting (1:1 binding  $RI\neq 0$ ,  $R_{max}=local$  for IL-2R  $\beta\gamma$ , apparent  $K_D$ , 1:1 binding  $RI=0$ ,  $R_{max}=local$  for IL-2R  $\alpha$ ). The respective  $K_D$  values are given in the table below.

Apparent $K_D$ [nM]	Hu IL-2R $\beta\gamma$	Hu IL-2R $\alpha$
4G8 IgG-IL-2 qm	5.9	No binding
4G8 Fab-IL-2 qm-Fab	10.4	No binding
28H1 IgG-IL-2 qm	6.2	No binding
28H1 Fab-IL-2 qm-Fab	11.4	No binding

The data show that the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates bind with at least as good affinity as the Fab-IL-2 qm-Fab immunoconjugates to the IL-2R  $\beta\gamma$  heterodimer, whereas they do not bind to the IL-2R  $\alpha$ -subunit due to the introduction of the mutations interfering with CD25 binding. Compared to the respective Fab-IL-2 qm-Fab immunoconjugates the affinity of the IgG-IL-2 qm fusion proteins appears to be slightly enhanced within the error of the method.

Similarly, the affinity of further constructs (4B9, DP47GS, 2B10, CH1A1A 98/99 2F1) comprising either IL-2 wt (see Example 4) or IL-2 qm to the IL-2R  $\beta\gamma$  heterodimer and the IL-2R  $\alpha$ -subunit was determined by SPR at 25° C. For all constructs the apparent  $K_D$  for the

human IL-2R  $\beta\gamma$  heterodimer was between 6 and 12 nM (irrespective of whether the construct comprises IL-2 wt or IL-2 qm), whereas only the constructs comprising IL-2 wt bind to the IL-2R  $\alpha$ -subunit at all ( $K_D$  for human IL-2R  $\alpha$  around 20 nM).

Biological Activity Assays with IgG-Cytokine Immunoconjugates

The biological activity of FAP-targeted 4G8-based IgG-IL-2 qm fusions was investigated in several cellular assays in comparison to commercially available IL-2 (Proleukin, Novartis/Chiron) and/or the Fab-IL-2-Fab immunoconjugates described in EP 11153964.9.

Binding to FAP Expressing Cells

Binding of FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate to human FAP expressed on stably transfected HEK293 cells was measured by FACS. Briefly, 250 000 cells per well were incubated with the indicated concentration of the immunoconjugate in a round-bottom 96-well plate, incubated for 30 min at 4° C., and washed once with PBS/0.1% BSA. Bound immunoconjugate was detected after incubation for 30 min at 4° C. with FITC-conjugated AffiniPure F(ab')<sub>2</sub> Fragment goat anti-human F(ab')<sub>2</sub> Specific (Jackson Immuno Research Lab #109-096-097, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) using a FACS CantoII (Software FACS Diva). The results are shown in FIG. 10. The data show that the IgG-IL-2 qm immunoconjugate binds to FAP-expressing cells with an EC50 value of 0.9 nM, comparable to that of the corresponding 4G8-based Fab-IL-2 qm-Fab construct (0.7 nM).

IFN- $\gamma$  Release by NK Cells (In Solution)

Subsequently, FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate was studied for the induction of IFN- $\gamma$  release by NK92 cells as induced by activation of IL-2R  $\beta\gamma$  signaling. Briefly, IL-2 starved NK92 cells (100 000 cells/well in 96-U-well plate) were incubated with different concentrations of IL-2 immunoconjugate, comprising quadruple mutant IL-2, for 24 h in NK medium (MEM alpha from Invitrogen (#22561-021) supplemented with 10% FCS, 10% horse serum, 0.1 mM 2-mercaptoethanol, 0.2 mM inositol and 0.02 mM folic acid). Supernatants were harvested and the IFN- $\gamma$  release was analysed using the anti-human IFN- $\gamma$  ELISA Kit II from Becton Dickinson (#550612). Proleukin (Novartis) and 28H1-based Fab-IL-2 qm-Fab served as positive control for IL-2-mediated activation of the cells. FIG. 11 shows that the FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate is equally efficacious in inducing IFN- $\gamma$  release as the affinity matured 28H1-based Fab-IL-2 qm-Fab immunoconjugate.

STATS Phosphorylation Assay

In a last set of experiments we studied the effects of the FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate on the induction of STATS phosphorylation compared to the 28H1 based Fab-IL-2-Fab and Fab-IL-2 qm-Fab immunoconjugates as well as Proleukin on human NK cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and T<sub>reg</sub> cells from human PBMCs. Briefly, blood from healthy volunteers was taken in heparin-containing syringes and PBMCs were isolated. PBMCs were treated with the indicated immunoconjugates at the indicated concentrations or with Proleukin (Novartis) as control. After 20 min incubation at 37° C., PBMCs were fixed with pre-warmed Cytotfix buffer (Becton Dickinson #554655) for 10 min at 37° C., followed by permeabilization with Phos-flow Perm Buffer III (Becton Dickinson #558050) for 30 min at 4° C. Cells were washed twice with PBS containing 0.1% BSA before FACS staining was performed using mixtures of flow cytometry antibodies for detection of

different cell populations and phosphorylation of STATS. Samples were analysed using a FACSCantoII with HTS from Becton Dickinson. NK cells were defined as CD3<sup>-</sup>CD56<sup>+</sup>, CD8 positive T cells were defined as CD3<sup>+</sup>CD8<sup>+</sup>, CD4 positive T cells were defined as CD4<sup>+</sup>CD25<sup>-</sup>CD127<sup>+</sup> and T<sub>reg</sub> cells were defined as CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>. For NK cells and CD8<sup>+</sup> T cells that show no or very low CD25 expression (meaning that IL-2R signaling is mediated primarily via the IL-2R  $\beta\gamma$  heterodimer) the results show that the 4G8-based IgG-IL-2 qm immunoconjugate was <10-fold less potent in inducing STATS phosphorylation than Proleukin, but slightly more potent than 28H1-based Fab-IL-2-Fab and Fab-IL-2 qm-Fab immunoconjugates. On CD4<sup>+</sup> T cells, that show a rapid up-regulation of CD25 upon stimulation, the 4G8-based IgG-IL-2 qm immunoconjugate was less potent than the 28H1Fab-IL-2-Fab immunoconjugate, but slightly more potent than the 28H1Fab-IL-2 qm-Fab immunoconjugate, and still showed induction of IL-2R signaling at saturating concentrations comparable to Proleukin and 28H1Fab-IL-2-Fab. This is in contrast to T<sub>reg</sub> cells where the potency of the 4G8-based IgG-IL-2 qm immunoconjugate was significantly reduced compared to the Fab-IL-2-Fab immunoconjugate due to the high CD25 expression on T<sub>reg</sub> cells and the low binding affinity of the 4G8-based IgG-IL-2 qm immunoconjugate to CD25. As a consequence of the abolishment of CD25 binding in the 4G8-based IgG-IL-2 qm immunoconjugate, IL-2 signaling in T<sub>reg</sub> cells is only activated via the IL-2R  $\beta\gamma$  heterodimer at concentrations where IL-2R signaling is activated on CD25-negative effector cells through the IL-2R  $\beta\gamma$  heterodimer. Taken together the 4G8-based IgG-IL-2 qm immunoconjugate described here is able to activate IL-2R signaling through the IL-2R  $\beta\gamma$  heterodimer, but does not result in a preferential stimulation of T<sub>reg</sub> cells over other effector cells. The results of these experiments are shown in FIG. 12.

**Binding of 2B10 IgG-IL-2 qm to TNC A2 Expressing Cells**  
Binding of TNC A2-targeted 2B10-based IgG-IL-2 qm immunoconjugate to human TNC A2 expressed on U87MG cells was measured by FACS. Briefly, 200 000 cells per well were incubated with the indicated concentration of the immunoconjugate in a round-bottom 96-well plate, incubated for 30 min at 4° C., and washed twice with PBS/0.1% BSA. Bound immunoconjugate was detected after incubation for 30 min at 4° C. with FITC-conjugated AffiniPure F(ab')<sub>2</sub> Fragment goat anti-human IgG Fc $\gamma$  Specific (Jackson Immuno Research Lab #109-096-098, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) using a FACS CantoII (Software FACS Diva). The results are shown in FIG. 13. The data show that the 2B10 IgG-IL-2 qm immunoconjugate binds to TNC A2-expressing U87MG cells equally well as the corresponding unconjugated IgG. Induction of NK92 Cell Proliferation by IgG-IL-2 Immunoconjugates

2B10 IgG-IL-2 qm, CH1A1A 98/99 2F1 IgG-IL-2 qm, CH1A1A 98/99 2F1 IgG-IL-2 wt, 4B9 IgG-IL-2 qm and 4B9 IgG-IL-2 wt immunoconjugates were tested for their ability to induce proliferation of NK92 cells. For proliferation assays, NK92 cells were starved in IL-2-free medium for 2 hours, 10000 cells/well seeded into a flat-bottom 96-well plate and then incubated for 3 days in a humidified incubator at 37° C., 5% CO<sub>2</sub> in the presence of the IL-2 immunoconjugates ( ). After 3 days, the ATP content of the cell lysates was measured using the CellTiter-Glo Luminescent Cell Viability Assay from Promega (#G7571/2/3). The percentage of growth was calculated setting a Proleukin (Novartis) concentration of 1.1 mg/ml to 100% proliferation and untreated cells without IL-2 stimulus to 0% prolifera-

tion. The results are shown in FIGS. 14 and 15. The data show that all constructs were able to induce NK92 cell proliferation, with the CH1A1A-based constructs being more active than the 2B10 IgG-IL-2 qm immunoconjugate, and the constructs comprising IL-2 wt being more active than the corresponding constructs with IL-2 qm.

### Example 3

In general, the P329G LALA mutations that almost completely abolish Fc $\gamma$ R interaction of human IgG<sub>1</sub> antibodies (see European patent application no. EP 11160251.2, incorporated herein by reference in its entirety) are introduced in order to reduce Fc $\gamma$ R binding/effector function and thus prevent excessive cytokine release when the respective cytokine receptors are co-activated with Fc $\gamma$ R signaling. In specific cases, for example when the antibody is targeting a highly tumor specific antigen, Fc effector functions may be retained by using an unmodified IgG Fc domain or may be even further enhanced via glycoengineering of the IgG Fc domain.

As an example thereof, we generated a CEA-targeted IgG-IL-2 qm immunoconjugate where one single IL-2 quadruple mutant was fused to the C-terminus of one heterodimeric heavy chain via a (SG<sub>4</sub>)<sub>3</sub>-linker based on the anti-CEA antibody clone CH1A1A. In this immunoconjugate the P329G LALA mutation was not included (see sequences of SEQ ID NOs 227, 229 and 231). The immunoconjugate was expressed and purified as human wildtype IgG- or glycoengineered IgG-IL-2 qm fusion protein as described below. Preparation of (Glycoengineered) IgG-IL-2 qm Immunoconjugate

CEA-targeted CH1A1A-based IgG-IL-2 qm immunoconjugate was produced by co-transfecting HEK293-EBNA cells with the mammalian antibody expression vectors. Exponentially growing HEK293-EBNA cells were transfected by the calcium phosphate method. Alternatively, HEK293 cells growing in suspension are transfected by polyethylenimine. For the production of unmodified non-glycoengineered IgG-IL-2 qm immunoconjugate, the cells were transfected only with antibody heavy and light chain expression vectors in a 1:1 ratio (wherein the antibody heavy chain vector is a 1:1 mixture of two vectors: a vector for the heavy chain with the effector moiety, and a vector for the heavy chain without effector moiety).

For the production of the glycoengineered CEA-targeted IgG-IL-2 qm immunoconjugate, the cells were co-transfected with two additional plasmids, one for expression of a GnTIII fusion polypeptide (a GnT-III expression vector), and one for mannosidase II expression (a Golgi mannosidase II expression vector) at a ratio of 4:4:1:1, respectively. Cells were grown as adherent monolayer cultures in T flasks using DMEM culture medium supplemented with 10% FCS, and were transfected when they are between 50 and 80% confluent. For the transfection of a T150 flask, 15 million cells were seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% v/v final), and cells were placed at 37° C. in an incubator with a 5% CO<sub>2</sub> atmosphere overnight. For each T150 flask to be transfected, a solution of DNA, CaCl<sub>2</sub> and water was prepared by mixing 94  $\mu$ g total plasmid vector DNA divided equally between the light and heavy chain expression vectors, water to a final volume of 469  $\mu$ l, and 469  $\mu$ l of a 1M CaCl<sub>2</sub> solution. To this solution, 938  $\mu$ l of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub> solution at pH 7.05 were added, mixed immediately for 10 sec and left to stand at room temperature for 20 sec. The suspension was diluted

with 10 ml of DMEM supplemented with 2% FCS, and added to the T150 flask in place of the existing medium. Then additional 13 ml of transfection medium were added. The cells were incubated at 37° C., 5% CO<sub>2</sub> for about 17 to 20 hours, before the medium was replaced with 25 ml DMEM, 10% FCS. The conditioned culture medium was harvested approximately 7 days after the media exchange by centrifugation for 15 min at 210×g. The solution was sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01% w/v was added, and kept at 4° C.

The secreted wildtype or glycoengineered CEA IgG-IL-2 qm immunoconjugates were purified from cell culture supernatants by affinity chromatography using Protein A affinity chromatography, followed by a size exclusion chromatographic step on a HiLoad Superdex 200 column (GE Healthcare) as described above. Protein concentration, purity, molecular weight, aggregate content and integrity were analysed as described above.

#### Oligosaccharide Structure Analysis of (Glycoengineered) IgG-IL-2 qm Immunoconjugates

For determination of the relative ratios of fucose-containing and non-fucosylated oligosaccharide structures, released glycans of purified immunoconjugate material are analyzed by MALDI TOF mass spectrometry. The immunoconjugate sample (about 50 µg) is incubated overnight at 37° C. with 5 mU N-glycosidase F (QAbio; PNGaseF: E-PNG01) in 2 mM Tris, pH 7.0, in order to release the oligosaccharide from the protein backbone. For deamination of glycans acetic acid to a final concentration of 150 mM is added and incubated for 1 h at 37° C. For analysis by MALDI TOF mass spectrometry, 2 µL of the sample are mixed on the MALDI target with 2 µL DHB matrix solution (2,5-dihydroxybenzoic acid [Bruker Daltonics #201346] dissolved in 50% ethanol/5 mM NaCl at 4 mg/ml) and analysed with MALDI TOF Mass Spectrometer Autoflex II instrument (Bruker Daltonics). Routinely, 50-300 shots are recorded and summed up to a single experiment. The spectra obtained are evaluated by the flex analysis software (Bruker Daltonics) and masses are determined for the each of the peaks detected. Subsequently, the peaks are assigned to fucose-containing or non-fucosylated carbohydrate structures by comparing the masses calculated and the masses theoretically expected for the respective structures (e.g. complex, hybrid and oligo- or high-mannose, respectively, with and without fucose).

For determination of the ratio of hybrid structures, the antibody samples are digested with N-glycosidase F and Endo-glycosidase H [QAbio; EndoH: E-EH02] concomitantly. N-glycosidase F releases all N-linked glycan structures (complex, hybrid and oligo- and high mannose structures) from the protein backbone and the Endo-glycosidase H cleaves all the hybrid type glycans additionally between the two N-acetylglucosamine (GlcNAc) residues at the reducing end of the glycan. This digest is subsequently treated and analysed by MALDI TOF mass spectrometry in the same way as described above for the N-glycosidase F digested sample. By comparing the pattern from the N-glycosidase F digest and the combined N-glycosidase F/Endo H digest, the degree of reduction of the signals of a specific carbohydrate structure is used to estimate the relative content of hybrid structures. The relative amount of each carbohydrate structure is calculated from the ratio of the peak height of an individual structure and the sum of the peak heights of all oligosaccharides detected. The amount of fucose is the percentage of fucose-containing structures related to all carbohydrate structures identified in the N-glycosidase F treated sample (e.g. complex, hybrid and oligo-

and high-mannose structures). The degree of non-fucosylation is the percentage of structures lacking fucose relative to all carbohydrates identified in the N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures).

#### Antibody-Dependent Cell-Mediated Cytotoxicity Assay

The wildtype and glycoengineered CEA-targeted CH1A1A IgG-IL-2 qm immunoconjugates were compared in ADCC assays for their potential to mediate antibody mediated cellular cytotoxicity. Briefly, CEA-overexpressing A549 human tumor cells as target cells were collected, washed and resuspended in culture medium, stained with freshly prepared Calcein AM (Molecular Probes) at 37° C. for 30 min, washed three times, counted and diluted to 300 000 cells/ml. This suspension was transferred to a round-bottom 96-well plate (30000 cells/well), the respective immunoconjugate dilution was added and incubated for 10 min to allow the binding of the tested immunoconjugate to the cells prior to contact with effector cells. Effector to target ratio was 25 to 1 for freshly isolated PBMCs. Co-incubation was performed for 4 hours. Two different read-out systems were used: the release of lactate dehydrogenase (LDH) into supernatant after disintegration of the attacked cells, and the retention of Calcein in the remaining living cells. LDH from co-culture supernatant was collected and analyzed with a LDH detection Kit (Roche Applied Science). Substrate conversion by the LDH enzyme was measured with an ELISA absorbance reader (SoftMaxPro software, reference wavelengths: 490 nm versus 650 nm). Residual Calcein in living cells was analyzed in a fluorescence reader (Wallac VICTOR3 1420 Multilabel COUNTER (Perkin Elmer)) after removing the rest of supernatant from pelletized cells, one washing step in PBS prior to lysis, and fixation of the cells by borate buffer (50 mM borate, 0.1% Triton).

FIG. 16 shows the result based on LDH detection. A similar result was obtained based on the calcein retention (not shown). Both the constructs were able to mediate ADCC, the glycoengineered construct being similarly active as the corresponding glycoengineered unconjugated IgG. As expected, the non-glycoengineered construct showed reduced activity as compared to the glycoengineered construct.

#### Example 4

FAP-targeted 28H1- or 4B9-based, CEA-targeted CH1A1A 98/99 2F1-based and non-targeted DP47GS-based IgG-IL-2 immunoconjugates were generated wherein one single wildtype IL-2 polypeptide is fused to the C-terminus of one heterodimeric heavy chain. Heterodimerization resulting in an immunoconjugate with a single IL-2 moiety was achieved by application of the knob-into-hole technology. In order to minimize the generation of homodimeric IgG-IL-2 fusions proteins the cytokine was fused to the knob-containing heavy chain (with deletion of the C-terminal Lys residue) via a G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> or a (G<sub>4</sub>S)<sub>3</sub> linker. The sequences of these immunoconjugates are given in SEQ ID NOs 193, 197 and 205 (28H1 with G<sub>4</sub>-(5G<sub>4</sub>)<sub>2</sub> linker) SEQ ID NOs 207, 273 and 211 (4B9 with (G<sub>4</sub>S)<sub>3</sub> linker), SEQ ID NOs 277, 279 and 283 (CH1A1A 98/99 2F1 with (G<sub>4</sub>S)<sub>3</sub> linker), SEQ ID NOs 219, 223 and 225 (DP47GS with G<sub>4</sub>-(SG<sub>4</sub>)<sub>2</sub> linker), SEQ ID NOs 219, 293 and 225 (DP47GS with (G<sub>4</sub>S)<sub>3</sub> linker). The antibody-cytokine fusion has IgG-like properties. To reduce FcγR binding/effector function and prevent FcR co-activation, P329G LALA mutations were introduced in the Fc domain. Both constructs were purified according to the methods described above. Final

purification was done by size exclusion chromatography (HiLoad 26/60 Superdex 200, GE Healthcare) in the final formulation buffer 20 mM histidine, 140 mM sodium chloride pH 6. FIGS. 17 to 20 show the respective chromatograms and elution profiles of the purification (A, B) as well as the analytical SDS-PAGE and size exclusion chromatographies of the final purified constructs (C, D). Yield was 15.6 mg/L for the untargeted DP47GS IgG-IL-2 immunoconjugate, 26.7 mg/ml for the 28H1 IgG-IL-2 immunoconjugate, 4.6 mg/L for the CH1A1A 98/99 2F1 IgG-IL-2 immunoconjugate and 11 mg/L for the 4B9 IgG-IL-2 immunoconjugate.

Subsequently, their binding properties to FAP, respectively lack of binding, as well as binding to IL-2R  $\beta\gamma$  and IL-2R  $\alpha$  chain were determined by SPR as described above (see Example 2). Cellular activity on immune effector cell populations and in vivo pharmacodynamic effects were also studied.

#### Example 5

FAP-targeted 4G8-based as well as TNC A2-targeted 2B10-based IgG-IL-10 immunoconjugates were constructed by fusing two different IL-10 cytokine formats to the C-terminus of the heavy chain of the heterodimeric IgG comprising a hole modification: either a single-chain IL-10 wherein a  $(G_4S)_4$  20-mer linker was inserted between two IL-10 molecules, or an engineered monomeric IL-10 (Josephson et al., J Biol Chem 275, 13552-7 (2000)). Both molecules were fused via a  $(G_4S)_3$  15-mer linker to the C-terminus of the heavy chain comprising a hole modification, with deletion of the C-terminal Lys residue. Heterodimerization resulting in only one heavy chain carrying an IL-10 moiety was achieved by application of the knob-into-hole technology. The IgG-cytokine fusion has IgG-like properties. To reduce Fc $\gamma$ R binding/effector function and prevent FcR co-activation, P329G LALA mutations were introduced in the Fc domain of the immunoconjugate. The sequences of the respective constructs are given in SEQ ID NOs 233, 235 and 239 (2B10 with scIL-10), SEQ ID NOs 233, 237 and 239 (2B10 with monomeric IL-10 "IL-10M1"), SEQ ID NOs 241, 243 and 205 (4G8 with scIL-10), SEQ ID NOs 241, 245 and 205 (4G8 with IL-10M1). All these immunoconjugates were purified according to the methods described above. Subsequently, their binding properties to FAP or TNC A2, respectively, as well as their affinities to human IL-10R1 were determined by SPR using the ProteOn XPR36 biosensor. Briefly, the targets FAP or TNC A2 as well as human IL-10R1 were immobilized in vertical orientation on the sensorchip surface (FAP by standard amine coupling, TNC A2 and human IL-10R1 (both biotinylated via a C-terminal avi-tag) by neutravidin-capture). Subsequently, the IgG-IL-10 immunoconjugates were injected in six different concentrations, including a zero-concentration, as analytes in horizontal orientation. After double-referencing, the sensorgrams were fit to a 1:1 interaction model to determine kinetic rate constants and affinities. The results from analytical SDS PAGE analysis and SPR-based affinity determinations to target antigens as well as IL-10 receptor are shown in FIGS. 21 and 22. The data show that the immunoconjugates bind to TNC A2 or FAP with  $K_D$  values of 52 or 26 pM, respectively, while  $K_D$  values for IL-10 receptor are 520 and 815 pM.

#### Example 6

According to the methods described above, IgG-cytokine fusion proteins were generated and expressed consisting of

one single 28H1-based or 4B9-based Fab region directed to FAP fused to the N-terminus of an Fc domain subunit comprising a hole modification, while the second Fab region of the IgG heavy chain with the knob modification was replaced by a cytokine moiety via a  $(G_4S)_n$  linker ( $n=1$ ). See FIG. 2C for a schematic representation of this immunoconjugate format (also referred to as "1+1" format). Cytokine moieties used were the IL-2 quadruple mutant described above and in PCT patent application no. PCT/EP2012/051991 (see SEQ ID NO: 3), IL-7 and IFN- $\alpha$ . Corresponding sequences of the fusion polypeptides comprising the cytokine moiety, fused to the N-terminus of an Fc domain subunit comprising a knob modification via a linker peptide, are given in SEQ ID NOs 247 (comprising quadruple mutant IL-2), 249 (comprising IL-7), and 251 (comprising IFN- $\alpha$ ). In these constructs, targeting of the immunoconjugate is achieved via the high affinity monovalent Fab region. This format may be recommended in cases where internalization of the antigen may be reduced using a monovalent binder. The immunoconjugates were produced, purified and analysed as described above. For constructs comprising IL-2 qm or IL-7, protein A affinity chromatography and size exclusion chromatography were combined in a single run. 20 mM histidine, 140 mM NaCl pH 6.0 was used as size exclusion chromatography and final formulation buffer. FIGS. 23-26 show the elution profiles and chromatograms of the purifications as well as the analytical SDS-PAGE and size exclusion chromatograms of the final purified constructs. The yields were 11 mg/L for the 4B9 "1+1" IgG-IL-2 qm, 43 mg/L for the 28H1 "1+1" IgG-IL-2 qm, 20.5 mg/L for the 4B9 "1+1" IgG-IL-7 and 10.5 mg/L for the 4B9 "1+1" IgG-IFN- $\alpha$  constructs.

The ability of "1+1" constructs comprising IL-2 qm to induce NK cell proliferation, compared to IgG-IL-2 qm immunoconjugates, was tested. NK-92 cells were starved for 2 h before seeding 10000 cells/well into 96-well-black-flat-clear bottom plates. The immunoconjugates were titrated onto the seeded NK-92 cells. After 72 h the ATP content was measured to determine the number of viable cells using the "CellTiter-Glo Luminescent Cell Viability Assay" Kit (Promega) according to the manufacturer's instructions. FIG. 27 shows that the "1+1" constructs are able to induce proliferation of NK-92 cells, being slightly less active than the corresponding IgG-IL-2 qm constructs.

The 4B9-based "1+1" constructs comprising IL-2 qm or IL-7 were tested for their ability to induce T cell proliferation, compared to IgG-IL-2 immunoconjugates. Peripheral blood mononuclear cells (PBMC) were prepared using His-topaque-1077 (Sigma Diagnostics Inc., St. Louis, Mo., USA). In brief, blood from buffy coats was diluted 5:1 with calcium- and magnesium-free PBS, and layered on His-topaque-1077. The gradient was centrifuged at 450 $\times$ g for 30 min at room temperature (RT) without breaks. The interphase containing the PBMCs was collected and washed three times with PBS (350 $\times$ g followed by 300 $\times$ g for 10 min at RT). PBMCs were pre-stimulated with 1  $\mu$ g/ml PHA-M (Sigma Aldrich #L8902) overnight, before they were labeled with 100 nM CFSE (carboxyfluorescein succinimidyl ester) for 15 min at 37 $^\circ$  C. Cells were washed with 20 ml medium before recovering the labeled PBMCs for 30 min at 37 $^\circ$  C. The cells were washed, counted, and 100000 cells were seeded into 96-well-U-bottom plates. The immunoconjugates were titrated onto the seeded cells for an incubation time of 6 days. Thereafter, cells were washed, stained for appropriate cell surface markers, and analyzed by FACS using a BD FACSCantoII. CD4 T cells were defined as CD3 $^+$ /CD8 $^-$ , and CD8 T cells as CD3 $^+$ /CD8 $^+$ .

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FIG. 28 shows that the “1+1” constructs comprising either IL-2 qm or IL-7 are able to induce proliferation of PHA-activated CD4 (A) and CD8 T cells (B). As for NK cells, the “1+1” construct comprising IL-2 qm is slightly less active than an IgG-IL-2 qm construct.

The 4B9-based “1+1” construct comprising IFN- $\alpha$  was tested for its ability to inhibit Daudi cell proliferation, in comparison to Roferon A (Roche). Briefly, Daudi cells were labeled with 100 nM CFSE and seeded into a 96-well U-bottom plate (50'000 cells/well). The molecules were added at the indicated concentrations, followed by incubation for 3 days at 37° C. Proliferation was measured by analyzing the CFSE dilution, excluding dead cells from analysis by use of life/dead stain.

FIG. 29 shows that the construct was able to inhibit proliferation of Daudi cells, at least as potently as Roferon A.

### Example 7

A single dose pharmacokinetics (PK) study was performed in tumor-free immunocompetent 129 mice for FAP-targeted IgG-IL2 immunoconjugates comprising either wild type or quadruple mutant IL-2, and untargeted IgG-IL-2 immunoconjugates comprising either wild type or quadruple mutant IL-2.

Female 129 mice (Harlan, United Kingdom), aged 8-9 weeks at the start of the experiment, were maintained under specific-pathogen-free conditions with daily cycles of 12 h light/12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2008016). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

Mice were injected i.v. once with FAP-targeted 28H1 IgG-IL2 wt (2.5 mg/kg) or 28H1 IgG-IL2 qm (5 mg/kg), or untargeted DP47GS IgG-IL2 wt (5 mg/kg) or DP47GS IgG-IL2 qm (5 mg/kg). All mice were injected i.v. with 200  $\mu$ l of the appropriate solution. To obtain the proper amount of immunoconjugate per 200  $\mu$ A, the stock solutions were diluted with PBS as necessary.

Mice were bled at 1, 8, 24, 48, 72, 96 h; and every 2 days thereafter for 3 weeks. Sera were extracted and stored at -20° C. until ELISA analysis. Immunoconjugate concentrations in serum were determined using an ELISA for quantification of the IL-2-immunoconjugate antibody (Roche-Penzberg). Absorption was measured using a measuring wavelength of 405 nm and a reference wavelength of 492 nm (VersaMax tunable microplate reader, Molecular Devices).

FIG. 30 shows the pharmacokinetics of these IL-2 immunoconjugates. Both the FAP-targeted (A) and untargeted (B) IgG-IL2 qm constructs have a longer serum half-life (approx. 30 h) than the corresponding IgG-IL-2 wt constructs (approx. 15 h). Of note, although the experimental conditions are not directly comparable, the serum half-life of the IL-2 immunoconjugates of the invention appears to be longer than the serum half-life of art-known “2+2” IgG-IL-2 immunoconjugates (see FIG. 1) as reported e.g. in Gillies et al., Clin Cancer Res 8, 210-216 (2002).

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Compound	Dose	Formulation buffer	Concentration (mg/mL)
28H1-IgG-IL2 wt	2.5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	3.84 (=stock solution)
28H1-IgG-IL2 qm	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	2.42 (=stock solution)
DP47GS-IgG-IL2wt	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	3.74 (=stock solution)
DP47GS-IgG-IL2QM	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	5.87 (=stock solution)

### Example 8

A biodistribution study was performed to assess tumor targeting of the immunoconjugates of the invention. FAP-targeted 28H1-based IgG-IL-2 qm was compared to FAP-targeted unconjugated 28H1 IgG and 4B9 IgG, and untargeted DP47GS IgG. Furthermore, a SPECT/CT imaging study was performed with 4B9 IgG-IL-2 qm, compared to DP47GS IgG-IL-2 qm, 4B9 IgG and DP47GS IgG.

#### DTPA Conjugation and <sup>111</sup>In Labeling

Solutions of 28H1 IgG-IL-2 qm, 28H1 IgG<sub>1</sub>, 4B9 IgG-IL-2 qm, 4B9 IgG<sub>1</sub> and DP47 IgG<sub>1</sub> were dialysed against phosphate buffered saline (PBS, 15 mM). Two mg of the constructs (5 mg/ml) were conjugated with isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclis, Dallas, Tex.) in 0.1 M NaHCO<sub>3</sub>, pH 8.2, under strict metal-free conditions, by incubation with a 5-fold molar excess of ITC-DTPA for one hour at room temperature (RT). Unconjugated ITC-DTPA was removed by dialysis against 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 5.5.

The purified conjugates were radiolabeled by incubation with <sup>111</sup>In (Covidien BV, Petten, The Netherlands) in 0.1 M MES buffer, pH 5.5 containing 0.05% bovine serum albumin (BSA) and 0.05% Tween-80, at RT, under strict metal-free conditions for 30 min. After radiolabeling ethylenediaminetetraacetic acid (EDTA) was added to a final concentration of 5 mM to chelate the unbound <sup>111</sup>In. The <sup>111</sup>In labeled products were purified by gel filtration on disposable G25M columns (PD10, Amersham Biosciences, Uppsala, Sweden). Radiochemical purity of purified <sup>111</sup>In labeled constructs were determined by instant thin-layer chromatography (ITLC) on TEC Control chromatography strips (Biodex, Shirley, N.Y.), using 0.1 M citrate buffer, pH 6.0, as the mobile phase. The specific activity of the <sup>111</sup>In-labeled preparations was 0.6-4.6 MBq/ $\mu$ g.

#### Lindmo Assay

The immunoreactive fraction of <sup>111</sup>In labeled antibody preparations was determined as described previously (Lindmo et al. (1984) J Immunol Methods 72, 77-89). Briefly, a serial dilution series of human embryonic kidney (HEK) cells transfected with fibroblast activation protein (FAP) cDNA (HEK-FAP cells) were incubated with 200 Bq of the <sup>111</sup>In-labeled construct at 37° C. for 1 hour. A duplicate of the lowest cell concentration was incubated in the presence of an excess of non-labeled construct to correct for non-specific binding. After incubation, the cells were washed, spun down and cell associated radioactivity was determined in the cell pellet in a gamma-counter (Wallac Wizzard 3" 1480 automatic  $\gamma$ -counter, Pharmacia LKB). The immunoreactive fraction of the preparations ranged between 75-94%.

## Animals

Female BALB/c nude mice (8-9 weeks, +/-20 g) were purchased from Janvier and housed in the Central Animal Facility of the Radboud University Nijmegen Medical Centre under standard conditions with 5 animals in individually ventilated cages with ad lib. access to food and water. After one week acclimatization the animals were inoculated s.c. with  $10 \times 10^6$  HEK-FAP cells in matrigel (1:3) in the left flank and optionally with  $5 \times 10^6$  HEK-293 cells in matrigel (1:3) in the right flank. Xenograft growth was monitored by caliper measurement ( $\text{volume} = (4/3 \cdot \pi) \cdot (1/2 \cdot \text{length}) \cdot (1/2 \cdot \text{width}) \cdot (1/2 \cdot \text{height})$ ). When xenografts reached a volume of  $100 \text{ mm}^3$ , mice were injected i.v. with the  $^{111}\text{In}$ -labeled constructs.

Biodistribution (28H1 IgG-IL-2 qm, 28H1 IgG<sub>1</sub>, 4B9 IgG<sub>1</sub> and DP47GS IgG<sub>1</sub>)

$^{111}\text{In}$ -labeled constructs (5 MBq, 150  $\mu\text{g}$ , 200  $\mu\text{l}$ ) were injected i.v. via the tail vein. Twenty-four hours after injection the animals were euthanized by suffocation in  $\text{CO}_2/\text{O}_2$  atmosphere. Blood, muscle, xenograft, lung, spleen, pancreas, kidney, stomach (empty), duodenum (empty) and liver were collected, weighed and radioactivity was determined in a gamma-counter (Wallac Wizard). Standards of the injected dose (1%) were counted simultaneously and tissue uptake was calculated as % of the injected dose per gram tissue (% ID/g).

SPECT-CT Analysis (4B9 IgG-IL-2 qm, 4B9 IgG<sub>1</sub>, DP47GS IgG-IL-2 qm and DP47GS IgG<sub>1</sub>)

$^{111}\text{In}$ -labeled 4B9-IgG-IL-2 qm, 4B9-IgG<sub>1</sub>, DP47GS-IgG-IL-2 qm and DP47GS-IgG<sub>1</sub> were injected i.v. (20 MBq, 50, 150, 300  $\mu\text{g}$ , 200  $\mu\text{l}$ ). At 4, 24, 72 and 144 hours after injection the animals were anesthetized with isoflurane/ $\text{O}_2$  and scanned for 30 to 60 min in a U-SPECT II microSPECT/CT camera (MILabs, Utrecht, The Netherlands) equipped with a 1.0 mm mouse collimator. Computed tomography (CT) was performed directly after SPECT. Both SPECT (voxel size of 0.4 mm) and CT scans were reconstructed with MILabs software and SPECT and CT scans were co-registered to determine exact location of radio-signal. 3D images were created using Siemens Inveon Research Workplace software.

FIG. 31 shows that there is no significant difference between tissue distribution and tumor targeting of 28H1 and 4B9 IgG<sub>1</sub> and 28H1 IgG-IL-2 qm at 24 hours (hence the cytokine does not significantly alter the tissue distribution and tumor targeting properties of the immunoconjugates), and that tumor-to-blood ratios for the FAP-targeted constructs are significantly greater than for the non-targeted DP47GS control IgG.

These results were confirmed in SPECT/CT imaging for the 4B9 IgG-IL-2 qm immunoconjugate (data not shown). 4B9 IgG-IL-2 qm localized in the FAP-positive HEK-FAP but not in the FAP-negative HEK-293 control tumors, while the untargeted DP47GS immunoconjugate did not localize in either tumor. Unlike with the unconjugated IgGs, a weak uptake of 4B9 IgG-IL-2 qm was observed also in the spleen.

## Example 9

Binding of 28H1-based IgG-IL-2 qm and a 28H1-based IgG-(IL-2 qm)<sub>2</sub> (i.e. a "2+2" format immunoconjugate as depicted in FIG. 1; sequences are shown in SEQ ID NOs 253 and 205) to NK 92 cells was compared. 200000 NK92 cells per well were seeded in a 96-well plate. The immunoconjugates were titrated onto the NK92 cells and incubated for 30 min at 4° C. to allow binding. The cells were washed twice with PBS containing 0.1% BSA to remove unbound

constructs. For detection of the immunoconjugates a FITC-labeled anti-human Fc-specific antibody was added for 30 min at 4° C. The cells were again washed twice with PBS containing 0.1% BSA and analyzed by FACS using a BD FACSCantoII.

As illustrated in FIG. 32, the "2+2" immunoconjugate shows better binding to NK 92 cells than the corresponding "2+1" construct.

## Example 10

## Induction of Human PBMC Proliferation by IL-2 Immunoconjugates

Peripheral blood mononuclear cells (PBMC) were prepared using Histopaque-1077 (Sigma Diagnostics Inc., St. Louis, Mo., USA). In brief, venous blood from healthy volunteers was drawn into heparinized syringes. The blood was diluted 2:1 with calcium- and magnesium-free PBS, and layered on Histopaque-1077. The gradient was centrifuged at  $450 \times g$  for 30 min at room temperature (RT) without breaks. The interphase containing the PBMCs was collected and washed three times with PBS ( $350 \times g$  followed by  $300 \times g$  for 10 min at RT).

Subsequently, PBMCs were labeled with 40 nM CFSE (carboxyfluorescein succinimidyl ester) for 15 min at 37° C. Cells were washed with 20 ml medium before recovering the labeled PBMCs for 30 min at 37° C. The cells were washed, counted, and 100000 cells were seeded into 96-well-U-bottom plates. Pre-diluted Proleukin (commercially available wild-type IL-2) or IL-2-immunoconjugates were titrated onto the seeded cells which were incubated for the indicated time points. After 4-6 days, cells were washed, stained for appropriate cell surface markers, and analyzed by FACS using a BD FACSCantoII. NK cells were defined as  $\text{CD}3^+/\text{CD}56^+$ , CD4 T cells as  $\text{CD}3^+/\text{CD}8^-$ , and CD8 T cells as  $\text{CD}3^+/\text{CD}8^+$ .

FIG. 33 shows proliferation of NK cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced NK cell proliferation in a concentration-dependent manner. Proleukin was more efficacious than the immunoconjugates at lower concentrations, this difference no longer existed at higher concentrations, however. At earlier time points (day 4), the IgG-IL2 constructs appeared slightly more potent than the Fab-IL2-Fab constructs. At later time points (day 6), all constructs had comparable efficacy, with the Fab-IL2 qm-Fab construct being least potent at the low concentrations.

FIG. 34 shows proliferation of CD4 T-cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced CD4 T cell proliferation in a concentration-dependent manner. Proleukin had a higher activity than the immunoconjugates, and the immunoconjugates comprising wild-type IL-2 were slightly more potent than the ones comprising quadruple mutant IL-2. As for the NK cells, the Fab-IL2 qm-Fab construct had the lowest activity. Most likely the proliferating CD4 T cells are partly regulatory T cells, at least for the wild-type IL-2 constructs.

FIG. 35 shows proliferation of CD8 T-cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced CD8 T cell proliferation in a concentration-dependent manner. Proleukin had a higher activity than the immunoconjugates, and the immunoconjugates comprising wild-type IL-2 were slightly more potent than the ones

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comprising quadruple mutant IL-2. As for the NK and CD4 T cells, the Fab-IL2 qm-Fab construct had the lowest activity.

## Example 11

## Proliferation and Activation Induced Cell Death of IL-2 Activated PBMCs

Freshly isolated PBMCs from healthy donors were pre-activated overnight with PHA-M at 1 µg/ml in RPMI1640 with 10% FCS and 1% Glutamine. After pre-activation PBMCs were harvested, labeled with 40 nM CFSE in PBS, and seeded in 96-well plates at 100 000 cells/well. Pre-activated PBMCs were stimulated with different concentrations of IL-2 immunoconjugates (4B9 IgG-IL-2 wt, 4B9 IgG-IL-2 qm, 4B9 Fab-IL-2 wt-Fab, and 4B9 Fab-IL-2 qm-Fab). After six days of IL-2 treatment PBMCs were treated with 0.5 µg/ml activating anti-Fas antibody overnight. Proliferation of CD4 (CD3<sup>+</sup>CD8<sup>-</sup>) and CD8 (CD3<sup>+</sup>CD8<sup>+</sup>) T cells was analyzed after six days by CFSE dilution. The percentage of living T cells after anti-Fas treatment was determined by gating on CD3<sup>+</sup> Annexin V negative living cells.

As shown in FIG. 36, all constructs induced proliferation of pre-activated T cells. At low concentrations the constructs comprising wild-type IL-2 wt were more active than the IL-2 qm-comprising constructs. IgG-IL-2 wt, Fab-IL-2 wt-Fab and Proleukin had similar activity. Fab-IL-2 qm-Fab was slightly less active than IgG-IL-2 qm. The constructs comprising wild-type IL-2 were more active on CD4 T cells than on CD8 T cells, most probably because of the activation of regulatory T cells. The constructs comprising quadruple mutant IL-2 were similarly active on CD8 and CD4 T cells.

As shown in FIG. 37, T cells stimulated with high concentrations of wild-type IL-2 are more sensitive to anti-Fas induced apoptosis than T cells treated with quadruple mutant IL-2.

## Example 12

The untargeted DP47GS construct (see SEQ ID NO: 299 and 297 for VH and VL sequences, respectively) was further characterized. As described above, conjugates of DP47GS IgG with wild-type or quadruple mutant IL-2 were made. These constructs showed similar binding to IL-2R and induction of immune cell (e.g. NK cell, CD8<sup>+</sup> cell and CD4<sup>+</sup> cell) proliferation in vitro as corresponding targeted constructs (data not shown). In contrast to immunoconjugates targeting a tumor antigen, however, they did not accumulate in tumor tissue (see Example 8).

A further pharmacokinetic study (in addition to the one shown in Example 7) was performed with the untargeted DP47GS IgG-IL-2 constructs comprising either wild-type or quadruple mutant IL-2. Male C57BL/6J mice (n=6 per group) were injected i.v. with 0.3, 1, 3 or 10 mg/kg DP47GS IgG-IL-2 wt or DP47GS IgG-IL-2 qm construct. The injection volume was 1 ml for all mice. Blood samples were taken at 2, 4, 8, 24, 48, 72, 96 and 168 hours after injection (from 3 mice at each time point) and stored at -20° C. until analysis. The constructs were quantified in the serum samples by ELISA, using anti-Fab antibodies for capturing and detection of the constructs. All samples and calibration standards were diluted 1:25 in mouse serum (obtained from Bioreclamation) prior to the analysis. Briefly, streptavidin-coated 96 well plates (Roche) were washed three times for 10 sec with PBS/0.05% Tween 20, before incubation with

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100 µl/well (0.5 µg/ml) biotinylated anti-human Fab anti-body (M-1.7.10; Roche Diagnostics) for 1 hour at room temperature. After washing the plate again three times with PBS/0.05% Tween 20, 50 µl/well of the serum samples or calibration standards and 50 µl/well PBS/0.5% BSA were added to give a final sample dilution of 1:50. Samples were incubated for 1 hour at room temperature, followed by washing the plate again three times with PBS/0.05% Tween 20. Next, the plate was incubated with 100 µl/well (0.5 µg/ml) digoxigenin-labeled anti-human Fab antibody (M-1.19.31; Roche Diagnostics) for 1 hour at room temperature, washed, incubated with 100 µl/well anti-digoxigenin POD (Roche Diagnostics Cat#11633716001) for 1 hour at room temperature, and washed again. Finally, 100 µl/well TMB peroxidase substrate (Roche Diagnostics Cat#11484281001) was added for about 5 minutes, before the substrate reaction was stopped with 50 µl/well 2N HCl. The plate was read within 2 minutes after stopping the reaction at 450 nm with a reference wavelength of 650 nm.

The result of this study is shown in FIG. 38. Both constructs showed long serum half life, with the construct comprising quadruple mutant IL-2 (B) being even longer lived than the one comprising wild-type IL-2 (A).

In addition, the lack of binding of DP47GS IgG to various proteins as well as human cells (PBMCs) was confirmed.

The binding specificity (or lack of such) of the DP47GS antibody was assessed in an ELISA-based test system with a panel of different unrelated antigens. The test was performed on 384 well MaxiSorp™ microtiter plates (Thermo Scientific Nunc, Cat#460372). After each incubation step the plates were washed three times with PBS/0.05% Tween-20. First, the different antigens, diluted in PBS, were coated on plates overnight at 6° C. The test concentrations and detailed information for the used antigens are listed in the table below.

Antigen	Source	Supplier	Cat#	Test concentration [µg/ml]
Histons	calf thymus	Roche Diagnostics	10223565601	2
BSA	bovine	Roche Diagnostics	10735108001	2
Fraction V	human	Roche Diagnostics	11376497001	2
Insulin	human	Roche Diagnostics	11376497001	2
Cardiolipin	bovine	Sigma-Aldrich	C1649	2
Heparin	porcine	Sigma-Aldrich	H9902	2
CD40 (hFc)	human	Sino Biological	1077-H03H	1
Parathyroid hormone aa 1-34 (PTH) (biotinylated)	human	AnaSpec	20690	0.5
dsDNA	calf thymus	Sigma-Aldrich	D4522	0.16
Hemocyanin	keyhole limpet	Sigma-Aldrich	H7017	0.22
Actin beta 2	human	Cytoskeleton	APHL99	0.67
Streptavidin	<i>Streptomyces avidinii</i>	Roche Diagnostics	11721674001	1
Gelatin	bovine	Roche Diagnostics	11111965001	2% blocking buffer diluted 1:600
<i>E. coli</i> lysate	<i>E. coli</i>	inhouse	—	—

Thereafter, the wells were blocked with 2% gelatin in water for 1 hour at room temperature (RT). The DP47GS

antibody (1 µg/ml in PBS) was incubated with the panel of captured antigens for 1.5 hours at RT. Bound antibody was detected using anti-human IgG antibody-HRP conjugate (GE Healthcare, Cat#9330V; diluted 1:1000 in PBS with 0.2% Tween-20 and 0.5% gelatin). After 1 hour incubation the plates were washed 6 times with PBS/0.05% Tween-20 and developed with freshly prepared BM blue POD substrate solution (BM blue: 3,3',5,5'-tetramethylbenzidine, Roche Diagnostics, Cat#11484281001) for 30 minutes at RT. Absorbance was measured at 370 nm. The blank value was defined without addition of antibody. An inhouse human IgG<sub>1</sub> antibody which exhibits unspecific binding to almost all of the captured antigens served as positive control.

The result of this experiment is shown in FIG. 39. The DP47GS antibody showed no binding to any of the captured antigens. The detected signals were in the range of the control samples without antibody.

Finally, the binding of the DP47GS antibody to human PBMCs was assessed. Since in the course of a typical immune response the combination of cell surface-presented proteins changes dramatically, binding was tested on PBMCs directly after isolation from healthy adults as well as after in vitro activation with two different stimuli.

Human PBMCs were isolated by Ficoll density gradient centrifugation from buffy coats or from heparinized fresh blood from healthy volunteers using Histopaque 1077 (Sigma-Aldrich, Germany). PBMCs were either directly subjected to binding assays (fresh PBMCs) or cultured and stimulated further. PBMCs were cultured at a cell density of 2×10<sup>6</sup> cells/ml in T cell medium consisting of RPMI 1640 (Gibco) supplemented with 10% (v/v) heat-inactivated FBS (PAA Laboratories), 1 mM sodium pyruvate (Sigma-Aldrich), 1% (v/v) L-alanyl-L-glutamine (Biochrom) and 10 nM β-mercaptoethanol (Sigma-Aldrich) at 37° C. For in vitro stimulation, Proleukin (200 U/ml, Novartis) and phytohaemagglutinin (PHA-L; 2 µg/mL, Sigma-Aldrich) were added during six days of cultivation (PHA-L activated PBMC). For in vitro re-stimulation, 6-well cell culture plates were coated with mouse anti-human CD3 (clone KT3, 1 µg/ml) and mouse anti-human CD28 antibodies (clone 28.2,

2 µg/ml, both from eBioscience) and PHA-L activated PBMC were added for additional 24 hours (re-stimulated PBMC). Binding of DP47GS antibody (with or without the L234A L235A (LALA) P329G mutation in the Fc domain) to cell surface proteins was monitored for a five-fold serial dilution series (highest concentration 200 nM) using a goat anti-human IgG Fc-specific secondary antibody conjugated to fluorescein isothiocyanate (FITC) (Jackson Laboratories) and flow cytometric analysis. All assays were performed at 4° C. to prevent internalization of surface proteins. Incubation of primary and secondary antibody was for 2 hours and for 1 hour, respectively. To allow simultaneous typing of leukocytes, combinations of fluorochrome-labeled mouse anti-human CD14, CD15, CD4, CD19 (all Biolegend), NKp46, CD3, CD56, CD8 (all BD Pharmingen) were added to the secondary antibody. Propidium iodide (1 µg/ml) was added directly before measurement on a FACSCantoII device running FACS Diva software (both BD Bioscience) to exclude permeable dead cells. Propidium iodide negative living cells were gated for T cells (CD14<sup>-</sup>CD3<sup>+</sup>CD4<sup>+</sup>/CD8<sup>+</sup>), B cells (CD14<sup>-</sup>CD19<sup>+</sup>), NK Cells (CD14<sup>-</sup>NKp46<sup>+</sup>/CD56<sup>+</sup>) or monocytes/neutrophils (CD3<sup>-</sup>CD56<sup>-</sup>CD14<sup>+</sup>/CD15<sup>+</sup>). The median FITC fluorescence of the various leukocyte types was determined as indicator for bound primary antibody and blotted against the primary antibody concentration using Prism4 (GraphPad Software).

As shown in FIG. 40, the DP47GS IgG antibody without Fc mutation showed binding only to Fcγ receptor bearing cells, e.g. NK cells and monocytes/neutrophils. No binding of DP47GS (LALA P329G) was detected on human PBMCs, regardless of their activation status. The LALA P329G mutation in the Fc domain completely abolished binding also to Fcγ receptor bearing cells.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

## SEQUENCE LISTING

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<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
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Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
85 90 95

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Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 100 105 110  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 115 120 125  
 Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser  
 130 135 140  
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 145 150 155 160  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 165 170 175  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 180 185 190  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
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<210> SEQ ID NO 2  
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 <220> FEATURE:  
 <223> OTHER INFORMATION: Human IL-2 (C125A)

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 35 40 45  
 Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys  
 50 55 60  
 Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu  
 65 70 75 80  
 Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu  
 85 90 95  
 Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala  
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 130

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Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu
   65                               70                               75                               80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
   85                               90                               95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
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<210> SEQ ID NO 4
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<212> TYPE: PRT
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35     40     45

Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
50     55     60

Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
65     70     75     80

Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys
85     90     95

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130    135    140

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165    170    175

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 Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser Val Pro  
                   290                                  295                                  300  
 Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
                   305                                  310                                  315                                  320  
 Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys  
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 Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln  
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 Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile  
                                   355                                  360                                  365  
 Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys  
                                   370                                  375                                  380  
 Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu  
                                   385                                  390                                  395                                  400  
 Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser  
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 Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met  
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 Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro  
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 Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu  
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 Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser  
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 Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile  
                                   485                                  490                                  495  
 Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met  
                                   500                                  505                                  510  
 Ser Tyr Leu Asn Ala Ser  
                                   515

<210> SEQ ID NO 5  
 <211> LENGTH: 340  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Single chain human IL-10

<400> SEQUENCE: 5

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro  
 1                  5                                  10                                  15  
 Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg  
                   20                                  25                                  30  
 Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu  
                   35                                  40                                  45  
 Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala  
                   50                                  55                                  60  
 Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala  
                   65                                  70                                  75                                  80  
 Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu  
                   85                                  90                                  95

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Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu  
 100 105 110  
 Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe  
 115 120 125  
 Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp  
 130 135 140  
 Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn  
 145 150 155 160  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
 165 170 175  
 Gly Gly Gly Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys  
 180 185 190  
 Thr His Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp  
 195 200 205  
 Ala Phe Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp  
 210 215 220  
 Asn Leu Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu  
 225 230 235 240  
 Gly Cys Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val  
 245 250 255  
 Met Pro Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn  
 260 265 270  
 Ser Leu Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys  
 275 280 285  
 His Arg Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val  
 290 295 300  
 Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met  
 305 310 315 320  
 Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met  
 325 330 335  
 Lys Ile Arg Asn  
 340

<210> SEQ ID NO 6  
 <211> LENGTH: 166  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Human IL-10 monomer (IL-10M1)

<400> SEQUENCE: 6

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro  
 1 5 10 15  
 Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg  
 20 25 30  
 Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu  
 35 40 45  
 Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala  
 50 55 60  
 Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala  
 65 70 75 80  
 Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu  
 85 90 95  
 Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu  
 100 105 110

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Pro Cys Glu Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val Glu  
 115 120 125

Gln Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys  
 130 135 140

Ala Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met  
 145 150 155 160

Thr Met Lys Ile Arg Asn  
 165

<210> SEQ ID NO 7  
 <211> LENGTH: 114  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Mutant human IL-15 (E53A, N79A)

<400> SEQUENCE: 7

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile  
 1 5 10 15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His  
 20 25 30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln  
 35 40 45

Val Ile Ser Leu Ala Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu  
 50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Ala Val  
 65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile  
 85 90 95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn  
 100 105 110

Thr Ser

<210> SEQ ID NO 8  
 <211> LENGTH: 19  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 8

Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly  
 1 5 10 15

Ala His Ser

<210> SEQ ID NO 9  
 <211> LENGTH: 57  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 9

atggactgga cctggagaat cctcttcttg gtggcagcag ccacaggagc ccactcc

57

<210> SEQ ID NO 10  
 <211> LENGTH: 57  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:

-continued

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<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 10

atggactgga cctggaggat cctcttcttg gtggcagcag ccacaggagc ccaactcc 57

<210> SEQ ID NO 11  
 <211> LENGTH: 22  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 11

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp  
 1 5 10 15

Phe Pro Gly Ala Arg Cys  
 20

<210> SEQ ID NO 12  
 <211> LENGTH: 66  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 12

atggacatga gggccccgc tcagctcctg ggcctcctgc tgctctggtt cccaggtgcc 60

aggtgt 66

<210> SEQ ID NO 13  
 <211> LENGTH: 19  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 13

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15

Val His Ser

<210> SEQ ID NO 14  
 <211> LENGTH: 57  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 14

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattcc 57

<210> SEQ ID NO 15  
 <211> LENGTH: 57  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 15

atgggctggt cctgcatcat cctgtttctg gtggctaccg ccaactggagt gcattcc 57

<210> SEQ ID NO 16  
 <211> LENGTH: 57  
 <212> TYPE: DNA

-continued

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: leader sequence

&lt;400&gt; SEQUENCE: 16

atgggctggt cctgcatcat cctgtttctg gtcgccacag ccaccggcgt gcactct 57

&lt;210&gt; SEQ ID NO 17

&lt;211&gt; LENGTH: 466

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Human IL-2R-beta-Fc(hole) fusion protein

&lt;400&gt; SEQUENCE: 17

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp  
1 5 10 15Phe Pro Gly Ala Arg Cys Ala Val Asn Gly Thr Ser Gln Phe Thr Cys  
20 25 30Phe Tyr Asn Ser Arg Ala Asn Ile Ser Cys Val Trp Ser Gln Asp Gly  
35 40 45Ala Leu Gln Asp Thr Ser Cys Gln Val His Ala Trp Pro Asp Arg Arg  
50 55 60Arg Trp Asn Gln Thr Cys Glu Leu Leu Pro Val Ser Gln Ala Ser Trp  
65 70 75 80Ala Cys Asn Leu Ile Leu Gly Ala Pro Asp Ser Gln Lys Leu Thr Thr  
85 90 95Val Asp Ile Val Thr Leu Arg Val Leu Cys Arg Glu Gly Val Arg Trp  
100 105 110Arg Val Met Ala Ile Gln Asp Phe Lys Pro Phe Glu Asn Leu Arg Leu  
115 120 125Met Ala Pro Ile Ser Leu Gln Val Val His Val Glu Thr His Arg Cys  
130 135 140Asn Ile Ser Trp Glu Ile Ser Gln Ala Ser His Tyr Phe Glu Arg His  
145 150 155 160Leu Glu Phe Glu Ala Arg Thr Leu Ser Pro Gly His Thr Trp Glu Glu  
165 170 175Ala Pro Leu Leu Thr Leu Lys Gln Lys Gln Glu Trp Ile Cys Leu Glu  
180 185 190Thr Leu Thr Pro Asp Thr Gln Tyr Glu Phe Gln Val Arg Val Lys Pro  
195 200 205Leu Gln Gly Glu Phe Thr Thr Trp Ser Pro Trp Ser Gln Pro Leu Ala  
210 215 220Phe Arg Thr Lys Pro Ala Ala Leu Gly Lys Asp Thr Gly Ala Gln Asp  
225 230 235 240Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
245 250 255Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
260 265 270Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
275 280 285Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
290 295 300Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
305 310 315 320

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys

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325	330	335
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu		
340	345	350
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys		
355	360	365
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu		
370	375	380
Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp		
385	390	395
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val		
405	410	415
Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp		
420	425	430
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His		
435	440	445
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro		
450	455	460
Gly Lys		
465		

&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 1401

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Human IL-2R-beta-Fc(hole) fusion protein

&lt;400&gt; SEQUENCE: 18

atggacatga ggggtcccg ctcagctctg ggcctcctgc tgctctgggt cccaggtgcc	60
agggtgtgagg tgaatggcac ttcccagttc acatgcttct acaactcgag agccaacatc	120
tcctgtgtct ggagccaaga tggggctctg caggacactt cctgccaaagt ccatgcctgg	180
ccggacagac ggcgggtggaa ccaaacctgt gagctgctcc ccgtgagtca agcatcctgg	240
gcctgcaacc tgatcctcgg agccccagat tctcagaaac tgaccacagt tgacatcgtc	300
accctgaggg tgctgtgcgg tgaggggggtg cgatggagggt tgatggccat ccaggacttc	360
aagccctttg agaaccctcg cctgatggcc cccatctccc tccaagttgt ccacgtggag	420
acccacagat gcaacataag ctgggaaatc tcccagcct cccactactt tgaaagacac	480
ctggagttcg agggccggac gctgtcccca ggccacacct gggaggaggc cccctgctg	540
actctcaagc agaagcagga atggatctgc ctggagacgc tcacccaga caccagtat	600
gagtttcagg tgcgggtcaa gcctctgcaa ggcgagttca cgacctggag cccctggagc	660
cagccctcgg ccttcagaac aaagcctgca gcccttgga aggacaccgg agctcaggac	720
aaaactcaca catgccacc gtgcccagca cctgaactcc tggggggacc gtcagtcttc	780
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc	840
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc	900
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt	960
gtggtcagcg tctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc	1020
aaggtctcca acaaaagcct cccagcccc atcgagaaaa ccatctccaa agccaaagg	1080
cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac	1140
caggctagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatcgc cgtggagtgg	1200

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gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccggtgt ggactccgac 1260
ggctccttct tcctcgtgag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1320
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1380
tccctgtctc cgggtaaatg a 1401

```

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<210> SEQ ID NO 19
<211> LENGTH: 492
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R-gamma-Fc(knob) fusion protein

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<400> SEQUENCE: 19

```

```

Met Leu Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu
1          5          10          15
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly
20          25          30
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp
35          40          45
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val
50          55          60
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro
65          70          75          80
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn
85          90          95
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr
100         105         110
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe
115         120         125
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln
130         135         140
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu
145         150         155         160
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn
165         170         175
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp
180         185         190
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe
195         200         205
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg
210         215         220
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp
225         230         235         240
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Pro Phe
245         250         255
Leu Phe Ala Leu Glu Ala Gly Ala Gln Asp Lys Thr His Thr Cys Pro
260         265         270
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275         280         285
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290         295         300
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305         310         315         320
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro

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```

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acacctgcc cccatgccgg 1200
gatgagctga ccaagaacca ggtcagcctg tggcgctgg tcaaaggctt ctatcccagc 1260
gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320
cccgctgtgg actccgacgg ctctctcttc ctctacagca agctcaccgt ggacaagagc 1380
aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440
tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

```

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<210> SEQ ID NO 21
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 21

```

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1      5      10      15
Val His Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala
20     25     30
Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu
35     40     45
Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu
50     55     60
Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys
65     70     75     80
Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro
85     90     95
Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln
100    105    110
Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro
115    120    125
Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln
130    135    140
Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly
145    150    155    160
Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr
165    170    175
Gln Pro Gln Leu Ile Cys Thr Gly Val Asp Glu Gln Leu Tyr Phe Gln
180    185    190
Gly Gly Ser Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp
195    200    205
His Glu Ala Arg Ala His His His His His
210    215

```

```

<210> SEQ ID NO 22
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 22

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```

atgggatgga gctgtatcat cctctctctg gtagcaacag ctaccggtgt gcattccgag 60
ctctgtgacg atgaccgcc agagatocca cagccacat tcaaagccat ggctacaag 120

```

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```

gaaggaacca tgttgaactg tgaatgcaag agaggtttcc gcagaataaa aagcgggtca 180
ctctatatgc tctgtacagg aaactctagc cactcgtcct gggacaacca atgtcaatgc 240
acaagctctg ccactcggaa cacaacgaaa caagtgcacac ctcaacctga agaacagaaa 300
gaaaggaaaa ccacagaaat gcaaagtcca atgcagccag tggaccaagc gagccttcca 360
ggtcactgca gggaaacctcc accatgggaa aatgaagcca cagagagaat ttatcatttc 420
gtggtggggc agatggttta ttatcagtgc gtccagggat acagggtctc acacagaggt 480
cctgctgaga gcgtctgcaa aatgacctac gggaagacaa ggtggacca gccccagctc 540
atatgcacag gtgtcgacga acagtatat tttcaggcg gctcaggcct gaacgacatc 600
ttcgaggccc agaagatcga gtggcacgag gctcgagctc accaccatca ccatcactga 660

```

```

<210> SEQ ID NO 23
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10; VL

```

```

<400> SEQUENCE: 23

```

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1             5             10            15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20            25            30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35            40            45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50            55            60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65            70            75            80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85            90            95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100           105

```

```

<210> SEQ ID NO 24
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10; VL

```

```

<400> SEQUENCE: 24

```

```

gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgctt gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
agggttcagc gcggtggatc cgggacagag ttactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttcgag aatggtctgc agccgcgcac gtttgccag 300
ggcaccaaag tcgagatcaa g 321

```

```

<210> SEQ ID NO 25
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10(GS); VL

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-continued

&lt;400&gt; SEQUENCE: 25

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1       5       10      15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20      25      30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35      40      45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50      55      60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65      70      75      80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85      90      95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100     105

```

&lt;210&gt; SEQ ID NO 26

&lt;211&gt; LENGTH: 321

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10(GS); VL

&lt;400&gt; SEQUENCE: 26

```

gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtcggaga ccggggtcacc      60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca      120
gggaaagccc ctaagcgctt gatctatgct gcatccagtt tgcagagtgg cgtcccatca      180
aggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct      240
gaagattttg ccacctatta ctgcttgtag aatggtctgc agcccgcgac gtttgccag      300
ggcaccaaag tcgagatcaa g                                     321

```

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 121

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10; VH

&lt;400&gt; SEQUENCE: 27

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1       5       10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20      25      30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35      40      45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50      55      60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100     105     110
Gln Gly Thr Thr Val Thr Val Ser Ser
115     120

```

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<210> SEQ ID NO 28  
 <211> LENGTH: 363  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10; VH

<400> SEQUENCE: 28

```

caggtgcaat tggtcgagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tca                                                                    363
  
```

<210> SEQ ID NO 29  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11; VL

<400> SEQUENCE: 29

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
          20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
          35          40          45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Asp Arg Phe Ser
          50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Tyr Thr Pro
          85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          100         105
  
```

<210> SEQ ID NO 30  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11; VL

<400> SEQUENCE: 30

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggt cctcatctat ggagcatcca gcagggccac tggcgtecca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtga ttactgtcag cagggtcagt atactcccc cacgttcggc      300
caggggacca aagtggaaat caaa                                              324
  
```

<210> SEQ ID NO 31

-continued

<211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11(VI); VL

<400> SEQUENCE: 31

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Tyr Thr Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 32  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11(VI); VL

<400> SEQUENCE: 32

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggt cctcatctat ggagcatcca gcagggccac tggcatccca 180  
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtcagt atactcccc cacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 33  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11; VH

<400> SEQUENCE: 33

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys  
 85 90 95

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Ala Lys Trp Arg Trp Met Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu  
                   100                  105                  110

Val Thr Val Ser Ser  
                   115

<210> SEQ ID NO 34  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11; VH

<400> SEQUENCE: 34

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac atggccgtat attactgtgc gaaatggaga	300
tggatgatgt ttgactactg gggccaagga accctggtea ccgtctcgag t	351

<210> SEQ ID NO 35  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11(MT); VH

<400> SEQUENCE: 35

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1                  5                  10                  15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	
20                  25                  30	
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35                  40                  45	
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val	
50                  55                  60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	
65                  70                  75                  80	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	
85                  90                  95	
Ala Lys Trp Arg Trp Met Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu	
100                  105                  110	
Val Thr Val Ser Ser	
115	

<210> SEQ ID NO 36  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F11(MT); VH

<400> SEQUENCE: 36

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240

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```
ctgcagatga acagcctgag agccgaggac accgccgtat attactgtgc gaaatggaga    300
tggatgatgt ttgactactg gggccaagga accctgggtca ccgtctcgag t          351
```

```
<210> SEQ ID NO 37
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3F2; VL
```

```
<400> SEQUENCE: 37
```

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Tyr Pro Gly
1             5             10             15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
                20             25             30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
            35             40             45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
            50             55             60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65             70             75             80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
            85             90             95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100            105
```

```
<210> SEQ ID NO 38
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3F2; VL
```

```
<400> SEQUENCE: 38
```

```
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt atccagggga aagagccacc    60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa    120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca    180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag    240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc    300
caggggacca aagtggaaat caaaa                                324
```

```
<210> SEQ ID NO 39
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3F2(YS); VL
```

```
<400> SEQUENCE: 39
```

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1             5             10             15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
                20             25             30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
            35             40             45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
            50             55             60
```

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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> SEQ ID NO 40  
<211> LENGTH: 324  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 3F2(YS); VL

<400> SEQUENCE: 40

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
ctctcttgca gggccagtc agtggttacc agtagctact tagcctggta ccagcagaaa 120  
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
cctgaagatt ttgcagtga ttactgtcag caggggtatta tgettccccc gacgttcggc 300  
caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 41  
<211> LENGTH: 117  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 3F2; VH

<400> SEQUENCE: 41

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15  
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
20 25 30  
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45  
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60  
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
65 70 75 80  
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95  
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
100 105 110  
Val Thr Val Ser Ser  
115

<210> SEQ ID NO 42  
<211> LENGTH: 351  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 3F2; VH

<400> SEQUENCE: 42

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct 120

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```

ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtgtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggtag 300
tttggtggtt ttaactactg gggccaagga accctgggtc ccgtctcgag t 351

```

```

<210> SEQ ID NO 43
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3D9, VL

```

```

<400> SEQUENCE: 43

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
                20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
            35          40          45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
            50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Leu Ile Pro
            85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100         105

```

```

<210> SEQ ID NO 44
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3D9, VL

```

```

<400> SEQUENCE: 44

```

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacagggttc gtggcagtag atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt tttactgtcag cagggtcagc ttattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

```

```

<210> SEQ ID NO 45
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3D9, VH

```

```

<400> SEQUENCE: 45

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20          25          30
Ala Met Ser Trp Val Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val

```

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35	40	45	
Ser Ala Ile Gly Val Ser Thr Gly Ser Thr Tyr Tyr Ala Asp Ser Val			
50	55	60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
65	70	75	80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu			
	100	105	110
Val Thr Val Ser Ser			
115			
<210> SEQ ID NO 46			
<211> LENGTH: 351			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: 3D9, VH			
<400> SEQUENCE: 46			
gagggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc		60	
tcctgtgcag cctccgatt caccttagc agttatgcta tgagctgggt ccgccagact		120	
ccagggaagg ggctggagtg ggtctcagct attggtgtta gtactggtag cacatactac		180	
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat		240	
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg		300	
ctgggtcctt ttgactactg gggccaagga accctgggtca ccgtctcgag t		351	
<210> SEQ ID NO 47			
<211> LENGTH: 117			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: 3D9(TA); VH			
<400> SEQUENCE: 47			
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly			
1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr			
	20	25	30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
	35	40	45
Ser Ala Ile Gly Val Ser Thr Gly Ser Thr Tyr Tyr Ala Asp Ser Val			
50	55	60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
65	70	75	80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu			
	100	105	110
Val Thr Val Ser Ser			
115			
<210> SEQ ID NO 48			
<211> LENGTH: 351			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			

<223> OTHER INFORMATION: 3D9(TA) ; VH

<400> SEQUENCE: 48

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctggggggtc cctgagactc	60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct	120
ccagggaagg ggtcggagtg ggtctcagct attggtgta gtactggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cagcgtgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg	300
ctgqgtcctt ttgactactg gggccaaqqa accctggtca ccqtctcqaq t	351

<210> SEQ ID NO 49

<211> LENGTH: 108

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: 4G8; VL

<400> SEQUENCE: 49

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser  
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
35 40 45

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro  
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> SEQ ID NO 50

<211> LENGTH: 324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: 4G8; VL

<400> SEQUENCE: 50

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtca gagtgttagc cgcagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggcatcca	180
gacagggtca gtggcagtggt atccgggacg gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagg ttattccccc taegtctggc	300
caqqqqacca aactqqaat caaa	324

<210> SEO ID NO 51

<211> LENGTH: 117

```
<211> LENGTH: 1
<212> TYPE: PRT
```

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: 4G8: VH

<400> SEQUENCE: 51

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> SEQ ID NO 52  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4G8; VH

<400> SEQUENCE: 52

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccgatt cacctttagc agttatgcca tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactcgg tgaagggcgg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg	300
ctgggtaatt ttgactactg gggccaagga accctggtea ccgtctcgag t	351

<210> SEQ ID NO 53  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B3; VL

<400> SEQUENCE: 53

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Tyr Gly Ala Tyr Ile Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 54

-continued

<211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B3; VL

<400> SEQUENCE: 54

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtgtagc agcaattact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggccgctaca tcagggccac tggeatccca      180
gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattccccc tacgttcggc      300
caggggacca aagtggaaat caaa                                           324
  
```

<210> SEQ ID NO 55  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B3; VH

<400> SEQUENCE: 55

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser
115
  
```

<210> SEQ ID NO 56  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B3; VH

<400> SEQUENCE: 56

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccgatt cacttttagc agttatgcca tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
ctgggtaatt ttgactactg gggccaagga accctggtea ccgtctcgag t              351
  
```

<210> SEQ ID NO 57  
 <211> LENGTH: 108

-continued

<212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4D6; VL

<400> SEQUENCE: 57

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Gln Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 58  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4D6; VL

<400> SEQUENCE: 58

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtc gagtgtagc agcaactact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggct cctcatccag ggcgcctcca gcagggccac tggcatccca 180  
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattccccc tacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 59  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4D6; VH

<400> SEQUENCE: 59

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu

-continued

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100	105	110	
Val Thr Val Ser Ser			
115			
<p>&lt;210&gt; SEQ ID NO 60            &lt;211&gt; LENGTH: 351            &lt;212&gt; TYPE: DNA            &lt;213&gt; ORGANISM: Artificial Sequence            &lt;220&gt; FEATURE:            &lt;223&gt; OTHER INFORMATION: 4D6; VH</p>			
<400> SEQUENCE: 60			
gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc			60
tcctgtgcag cctccggatt caccttagc agttatgcc tgaagctgggt ccgccaggct			120
ccaggggaagg ggtcggagtg ggtctcagct attagtggta gtggtggtag cacatactac			180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat			240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg			300
ctgggtaatt ttgactactg gggccaagga accctgggtc ccgtctcag t			351
<p>&lt;210&gt; SEQ ID NO 61            &lt;211&gt; LENGTH: 108            &lt;212&gt; TYPE: PRT            &lt;213&gt; ORGANISM: Artificial Sequence            &lt;220&gt; FEATURE:            &lt;223&gt; OTHER INFORMATION: 2C6; VL</p>			
<400> SEQUENCE: 61			
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly			
1 5 10 15			
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser			
20 25 30			
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu			
35 40 45			
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser			
50 55 60			
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu			
65 70 75 80			
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Gln Ile Pro			
85 90 95			
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys			
100 105			
<p>&lt;210&gt; SEQ ID NO 62            &lt;211&gt; LENGTH: 324            &lt;212&gt; TYPE: DNA            &lt;213&gt; ORGANISM: Artificial Sequence            &lt;220&gt; FEATURE:            &lt;223&gt; OTHER INFORMATION: 2C6; VL</p>			
<400> SEQUENCE: 62			
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc			60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa			120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca			180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag caggctggag			240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagc agattcccc tacgttcggc			300
caggggacca aagtggaaat caaa			324

-continued

<210> SEQ ID NO 63  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2C6; VH

<400> SEQUENCE: 63

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Ala Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Phe Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser
115
```

<210> SEQ ID NO 64  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2C6; VH

<400> SEQUENCE: 64

```
gagggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatc cacctttagc agttatgcc tgcagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggga gtgctgggta tacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg      300
tttggaatt ttgactactg gggccaagga accctggtca ccgtctcgag t                  351
```

<210> SEQ ID NO 65  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 5H5; VL

<400> SEQUENCE: 65

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1          5          10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35          40          45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50          55          60
```

-continued

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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro  
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> SEQ ID NO 66  
<211> LENGTH: 324  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5H5; VL

<400> SEQUENCE: 66

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggt cctcatctat ggagcatcca gcagggccac tggcatccca	180
gacagggtta gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgt ttactgtcag cagggtaatc agattcccc tacgttcggt	300
caggggacca aagtggaaat caaa	324

<210> SEQ ID NO 67  
<211> LENGTH: 116  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5H5; VH

<400> SEQUENCE: 67

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	
20 25 30	
Thr Met Ser Trp Val Arg Arg Ser Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
Ser Ala Ile Ser Gly Gly Gly Arg Thr Tyr Tyr Ala Asp Ser Val Lys	
50 55 60	
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu	
65 70 75 80	
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala	
85 90 95	
Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val	
100 105 110	
Thr Val Ser Ser	
115	

<210> SEQ ID NO 68  
<211> LENGTH: 348  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5H5; VH

<400> SEQUENCE: 68

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctggggggtc cctgagactc	60
tcctgtgcag cctccgatt caccttagc agttatacca tgagctgggt ccgccggtct	120

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```

ccaggggaagg ggctggagtg ggtctcagct attagtgggtg gtggtaggac atactacgca 180
gactccgtga agggcccggtt caccatctcc agagacaatt ccaagaacac gctgtatctg 240
cagatgaaca gcctgagagc cgaggacacg gccgtatatt actgtgcgaa aggttggttt 300
acgccttttg actactgggg ccaaggaacc ctggtcaccg tctcgagt 348

```

```

<210> SEQ ID NO 69
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2C4; VL

```

```

<400> SEQUENCE: 69

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35          40          45
Ile Tyr Gly Ala Ser Ile Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro
85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100         105

```

```

<210> SEQ ID NO 70
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2C4; VL

```

```

<400> SEQUENCE: 70

```

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtc gagtgtagc agtaactact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggtgcctcca ttagggccac tggcatccca 180
gacagggtta gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtaatc agattcccc tacgttcggt 300
caggggacca aagtggaaat caaa 324

```

```

<210> SEQ ID NO 71
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2C4; VH

```

```

<400> SEQUENCE: 71

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45

```

-continued

---

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu  
100 105 110

Val Thr Val Ser Ser  
115

<210> SEQ ID NO 72  
<211> LENGTH: 351  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 2C4; VH

<400> SEQUENCE: 72

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctggggggtc cctgagactc 60  
tccctgtgcag cctccggatt cacctttagc agttatgccg tgagctgggt ccgccaggct 120  
ccagggaagg ggctggagtg ggtctcagct attagcggta gtggtggtag cacatactac 180  
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cagctgtgat 240  
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300  
tttacgcctt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> SEQ ID NO 73  
<211> LENGTH: 108  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 2D9; VL

<400> SEQUENCE: 73

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser  
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro  
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> SEQ ID NO 74  
<211> LENGTH: 324  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 2D9; VL

<400> SEQUENCE: 74

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```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttaactgtcag cagggtaatc agattccccc tacgttcggt      300
caggggacca aagtggaaat caaa                                           324

```

```

<210> SEQ ID NO 75
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2D9; VH

```

```

<400> SEQUENCE: 75

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser
115

```

```

<210> SEQ ID NO 76
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2D9; VH

```

```

<400> SEQUENCE: 76

```

```

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagcggt a gtggtggtag cacatactac      180
gcagactccg tgaagggccg gtccaccatc tccagagaca attccaagaa cagctgtgat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg      300
tttacgcctt ttgactactg gggccaagga accctgggtc ccgtctcgag t              351

```

```

<210> SEQ ID NO 77
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4B8; VL

```

```

<400> SEQUENCE: 77

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly

```

-continued

1	5	10	15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser	20	25	30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu	35	40	45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser	50	55	60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu	65	70	75
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro	85	90	95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	100	105	

<210> SEQ ID NO 78  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B8; VL

<400> SEQUENCE: 78

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggt cctcatctat ggagcatcca gcagggccac tggcatccca	180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattccccc tacgttcggc	300
caggggacca aagtggaaat caaa	324

<210> SEQ ID NO 79  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B8; VH

<400> SEQUENCE: 79

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	20	25	30	
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	35	40	45	
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val	50	55	60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	65	70	75	80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	85	90	95	
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu	100	105	110	
Val Thr Val Ser Ser	115			

<210> SEQ ID NO 80  
 <211> LENGTH: 351

-continued

<212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B8; VH

<400> SEQUENCE: 80

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctggggggtc cctgagactc      60
tcctgtgcag cctccgatt cacttttagc agttatgcca tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
ctgggtaatt ttgactactg gggccaagga accctggtea ccgtctcgag t              351
  
```

<210> SEQ ID NO 81  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 7A1; VL

<400> SEQUENCE: 81

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10           15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35          40          45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Gln Ile Pro
85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100         105
  
```

<210> SEQ ID NO 82  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 7A1; VL

<400> SEQUENCE: 82

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggeatccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagc agattcccc tacgttcggc      300
caggggacca aagtggaaat caaa                                324
  
```

<210> SEQ ID NO 83  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 7A1; VH

-continued

&lt;400&gt; SEQUENCE: 83

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Phe Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

&lt;210&gt; SEQ ID NO 84

&lt;211&gt; LENGTH: 351

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 7A1; VH

&lt;400&gt; SEQUENCE: 84

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
 tcctgtgcag cctccgatt cacctttagc agttatgccg tgagctgggt ccgccaggct 120  
 ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180  
 gcagactcgc tgaagggcgc gttcaccatc tccagagaca attccaagaa cacgctgtat 240  
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300  
 tttgggaatt ttgactactg gggccaagga accctggtea ccgtctcgag t 351

&lt;210&gt; SEQ ID NO 85

&lt;211&gt; LENGTH: 108

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 13C2; VL

&lt;400&gt; SEQUENCE: 85

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Leu Ile Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

-continued

<210> SEQ ID NO 86  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13C2; VL

<400> SEQUENCE: 86

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggeatccca      180
gacagggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtga ttactgtcag cagggtcagc ttattcccc tacgttcggc      300
caggggacca aagtggaaat caaa                                           324
  
```

<210> SEQ ID NO 87  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13C2; VH

<400> SEQUENCE: 87

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1             5             10             15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20            25            30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35            40            45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50            55            60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65            70            75            80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85            90            95
Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100           105           110
Val Thr Val Ser Ser
115
  
```

<210> SEQ ID NO 88  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13C2; VH

<400> SEQUENCE: 88

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct      120
ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg      300
ctgggtcctt ttgactactg gggccaagga accctggtea ccgtctcgag t                                           351
  
```

-continued

<210> SEQ ID NO 89  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13E8; VL

<400> SEQUENCE: 89

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Leu Asn Ile Pro  
 85 90 95  
 Ser Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 90  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13E8; VL

<400> SEQUENCE: 90

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtca gactgttagc agcagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180  
 gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtga ttactgtcag caggggtctga atattccctc gacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 91  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13E8; VH

<400> SEQUENCE: 91

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

-continued

---

85	90	95
Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu		
100	105	110
Val Thr Val Ser Ser		
115		

<210> SEQ ID NO 92  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 13E8; VH

<400> SEQUENCE: 92

gagggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccggatt cacccttagc agttatgcc tgaagctgggt ccgccaggct	120
ccagggaagg ggtcggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg	300
ttgggtccgt ttgactactg gggccaagga accctggtea ccgtctcgag t	351

<210> SEQ ID NO 93  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14C10; VL

<400> SEQUENCE: 93

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly	
1 5 10 15	
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser	
20 25 30	
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu	
35 40 45	
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser	
50 55 60	
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu	
65 70 75 80	
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly His Ile Ile Pro	
85 90 95	
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
100 105	

<210> SEQ ID NO 94  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14C10; VL

<400> SEQUENCE: 94

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca	180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag	240

-continued

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```
cctgaagatt ttgcagtgtg ttactgtcag cagggtcata ttattcccc gacgttcggc 300
caggggacca aagtggaaat caaaa 324
```

```
<210> SEQ ID NO 95
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 14C10; VH
```

```
<400> SEQUENCE: 95
```

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20        25        30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35        40        45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50        55        60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65        70        75        80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85        90        95
Ala Lys Ala Trp Met Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100       105       110
Val Thr Val Ser Ser
115
```

```
<210> SEQ ID NO 96
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 14C10; VH
```

```
<400> SEQUENCE: 96
```

```
gagggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgccg tgagctgggt ccgccaggct 120
ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagcttgg 300
atggggcctt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351
```

```
<210> SEQ ID NO 97
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 17A11; VL
```

```
<400> SEQUENCE: 97
```

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1          5          10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20        25        30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35        40        45
```

-continued

---

```

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50                      55                      60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                      70                      75                      80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Leu Asn Ile Pro
                      85                      90                      95

Ser Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
      100                      105

```

```

<210> SEQ ID NO 98
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 17A11; VL

```

```

<400> SEQUENCE: 98

```

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca      180
gacagggtta gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag caggggtctga atattccctc gacgttcggc      300
caggggacca aagtggaaat caaa                                           324

```

```

<210> SEQ ID NO 99
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 17A11; VH

```

```

<400> SEQUENCE: 99

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5                      10                      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                      45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
      50                      55                      60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                      70                      75                      80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                      90                      95

Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
      100                      105                      110

Val Thr Val Ser Ser
      115

```

```

<210> SEQ ID NO 100
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 17A11; VH

```

```

<400> SEQUENCE: 100

```

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60

```

-continued

---

```

tctctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct 120
ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300
ttgggtccgt ttgactactg gggccaagga accctggtea ccgtctcgag t 351

```

```

<210> SEQ ID NO 101
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 19G1; VL

```

```

<400> SEQUENCE: 101

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1             5             10            15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20            25            30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35            40            45
Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50            55            60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65            70            75            80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85            90            95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100           105

```

```

<210> SEQ ID NO 102
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 19G1; VL

```

```

<400> SEQUENCE: 102

```

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

```

```

<210> SEQ ID NO 103
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 19G1; VH

```

```

<400> SEQUENCE: 103

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1             5             10            15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20            25            30

```

-continued

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ile Ser Ser Gly Gly Leu Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> SEQ ID NO 104  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 19G1; VH

<400> SEQUENCE: 104

gaggtgcaat tgttgaggatc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
 tcctgtgcag cctccggatt cacctttagc agttatgcga tgagctgggt ccgccaggct 120  
 ccagggaagg ggctggagtg ggtctcagcg attattagta gtggtggtct cacatactac 180  
 gcagactcgg tgaaggcccg gtccaccatc tccagagaca attccaagaa cacgctgtat 240  
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300  
 tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

<210> SEQ ID NO 105  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 20G8; VL

<400> SEQUENCE: 105

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 106  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 20G8; VL

-continued

&lt;400&gt; SEQUENCE: 106

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca      180
gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttaactgtcag cagggtatta tgcttcccc gacgttcggc      300
caggggacca aagtggaaat caaa                                           324

```

&lt;210&gt; SEQ ID NO 107

&lt;211&gt; LENGTH: 117

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 20G8; VH

&lt;400&gt; SEQUENCE: 107

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ile Gly Ser Gly Ser Arg Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser
115

```

&lt;210&gt; SEQ ID NO 108

&lt;211&gt; LENGTH: 351

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 20G8; VH

&lt;400&gt; SEQUENCE: 108

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcaa tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attattggga gtggtagtcg tacatactac      180
gcagactccg tgaagggccg gtccaccatc tccagagaca attccaagaa cagctgtgat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c          351

```

&lt;210&gt; SEQ ID NO 109

&lt;211&gt; LENGTH: 108

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9; VL

-continued

&lt;400&gt; SEQUENCE: 109

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

&lt;210&gt; SEQ ID NO 110

&lt;211&gt; LENGTH: 324

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9; VL

&lt;400&gt; SEQUENCE: 110

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
 gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtatta tgettcccc gacgttcggc 300  
 caggggacca aagtggaaat caaa 324

&lt;210&gt; SEQ ID NO 111

&lt;211&gt; LENGTH: 117

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9; VH

&lt;400&gt; SEQUENCE: 111

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

-continued

<210> SEQ ID NO 112  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B9; VH

<400> SEQUENCE: 112

```

gaggtgcaat tgttgagtc tgggggagc ttggtacagc ctggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attattgcta gtggtgctag cacatactac      180
gcagactccg tgaaggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctggtea ccgtctcgtc c              351

```

<210> SEQ ID NO 113  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 5B8; VL

<400> SEQUENCE: 113

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1         5             10             15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20        25             30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35        40             45
Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50        55             60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65        70             75             80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85        90             95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100       105

```

<210> SEQ ID NO 114  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 5B8; VL

<400> SEQUENCE: 114

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtga ttactgtcag cagggtatta tgcttcccc gacgttcggc      300
caggggacca aagtggaaat caaa              324

```

<210> SEQ ID NO 115  
 <211> LENGTH: 117  
 <212> TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 5B8; VH

&lt;400&gt; SEQUENCE: 115

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Trp Gly Gly Gly Arg Ser Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

&lt;210&gt; SEQ ID NO 116

&lt;211&gt; LENGTH: 351

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 5B8; VH

&lt;400&gt; SEQUENCE: 116

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
 tcctgtgcag cctccgatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120  
 ccagggaagg ggctggagtg ggtctcagct atttggggtg gtggtcgtag cacatactac 180  
 gcagactcgc tgaagggcgc gttcaccatc tccagagaca attccaagaa cacgctgtat 240  
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300  
 tttggtggtt ttaactactg gggccaagga accctggtea ccgtctcgtc c 351

&lt;210&gt; SEQ ID NO 117

&lt;211&gt; LENGTH: 108

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 5F1; VL

&lt;400&gt; SEQUENCE: 117

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
 85 90 95

-continued

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> SEQ ID NO 118  
<211> LENGTH: 324  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5F1; VL

<400> SEQUENCE: 118

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa 120  
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
cctgaagatt ttgcagtga ttactgtcag caggggtatta tgettccccc gacgttcggc 300  
caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 119  
<211> LENGTH: 117  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5F1; VH

<400> SEQUENCE: 119

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15  
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
20 25 30  
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45  
Ser Ala Ile Ile Ser Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60  
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
65 70 75 80  
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95  
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
100 105 110  
Val Thr Val Ser Ser  
115

<210> SEQ ID NO 120  
<211> LENGTH: 351  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 5F1; VH

<400> SEQUENCE: 120

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120  
ccaggaagg ggctggagtg ggtctcagct attattagta gtggggctag cacatactac 180  
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240  
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300

-continued

tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c 351

<210> SEQ ID NO 121  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14B3; VL

<400> SEQUENCE: 121

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 122  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14B3; VL

<400> SEQUENCE: 122

gaaatcggtg taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggt cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
 gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtga ttactgtcag cagggtatta tgcttcccc gacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 123  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14B3; VH

<400> SEQUENCE: 123

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Leu Ala Ser Gly Ala Ile Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

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65	70	75	80
Leu Gln Met Asn Ser	Leu Arg Ala Glu Asp Thr	Ala Val Tyr Tyr Cys	
	85	90	95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu			
	100	105	110
Val Thr Val Ser Ser			
	115		

<210> SEQ ID NO 124  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 14B3; VH

<400> SEQUENCE: 124

gagggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccggatt caccttagc agttatgcta tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attttggcta gtggtgcgat cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg	300
tttggtggtt ttaactactg gggccaagga accctggtea ccgtctcgtc c	351

<210> SEQ ID NO 125  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 16F1; VL

<400> SEQUENCE: 125

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly	
1	15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser	
20	30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu	
35	45
Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser	
50	60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu	
65	80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro	
85	95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
100	105

<210> SEQ ID NO 126  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 16F1; VL

<400> SEQUENCE: 126

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatecca	180

-continued

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gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

```

```

<210> SEQ ID NO 127
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 16F1; VH

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<400> SEQUENCE: 127

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5      10      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20     25     30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35     40     45
Ser Gly Ile Ile Gly Ser Gly Gly Ile Thr Tyr Tyr Ala Asp Ser Val
50     55     60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65     70     75     80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85     90     95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100    105    110
Val Thr Val Ser Ser
115

```

```

<210> SEQ ID NO 128
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 16F1; VH

```

```

<400> SEQUENCE: 128

```

```

gagggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120
ccaggggaagg ggctggagtg ggtctcaggt attattggta gtggtgggtat cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

```

```

<210> SEQ ID NO 129
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 16F8; VL

```

```

<400> SEQUENCE: 129

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1      5      10      15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20     25     30

```

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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
           35                          40                          45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
           50                          55                          60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
           65                          70                          75                          80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
                           85                          90                          95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
                           100                          105

<210> SEQ ID NO 130  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 16F8; VL

<400> SEQUENCE: 130

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
 gacagggtta gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 131  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 16F8; VH

<400> SEQUENCE: 131

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1                  5                          10                          15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
           20                          25                          30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
           35                          40                          45  
 Ser Ala Ile Leu Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
           50                          55                          60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65                          70                          75                          80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
           85                          90                          95  
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
           100                          105                          110  
 Val Thr Val Ser Ser  
           115

<210> SEQ ID NO 132  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 16F8; VH

-continued

&lt;400&gt; SEQUENCE: 132

```

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgccg tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attcttggtg gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctgggtc cagtctcgtc c              351

```

&lt;210&gt; SEQ ID NO 133

&lt;211&gt; LENGTH: 108

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: O3C9; VL

&lt;400&gt; SEQUENCE: 133

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1             5             10            15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20            25            30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35            40            45
Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50            55            60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65            70            75            80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85            90            95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100           105

```

&lt;210&gt; SEQ ID NO 134

&lt;211&gt; LENGTH: 324

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: O3C9; VL

&lt;400&gt; SEQUENCE: 134

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtcg gagtgttacc agtagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc      300
caggggacca aagtggaaat caaaa                                324

```

&lt;210&gt; SEQ ID NO 135

&lt;211&gt; LENGTH: 117

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: O3C9; VH

&lt;400&gt; SEQUENCE: 135

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1             5             10            15

```

-continued

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe  
                   20                  25                  30

Ala Met Ser Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val  
                   35                  40                  45

Ser Ala Ile Ile Gly Ser Gly Ser Asn Thr Tyr Tyr Ala Asp Ser Val  
                   50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
                   65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
                   85                  90                  95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
                   100                  105                  110

Val Thr Val Ser Ser  
                   115

<210> SEQ ID NO 136  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: O3C9; VH

<400> SEQUENCE: 136

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
 tcctgtgcag cctccggatt cacctttagc agttttgccg tgagctgggt ccgtcagtct 120  
 ccagggaagg ggctggagtg ggtctcagct attattggtg gtggtagtaa cacatactac 180  
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cgcgtgtgat 240  
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300  
 tttggtgggt ttaactactg gggccaagga accctgggtc cgcgtctcgtc c 351

<210> SEQ ID NO 137  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: O2D7; VL

<400> SEQUENCE: 137

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1                  5                  10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
                   20                  25                  30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
                   35                  40                  45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Thr Pro Asp Arg Phe Ser  
                   50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
                   65                  70                  75                  80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ala Ile Met Leu Pro  
                   85                  90                  95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
                   100                  105

<210> SEQ ID NO 138  
 <211> LENGTH: 324  
 <212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: O2D7; VL

<400> SEQUENCE: 138

```

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcacccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag caggctatta tgcttcctcc gacgttcggc      300
caggggacca aagtggaaat caaa                                           324
  
```

<210> SEQ ID NO 139  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: O2D7; VH

<400> SEQUENCE: 139

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser
115
  
```

<210> SEQ ID NO 140  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: O2D7; VH

<400> SEQUENCE: 140

```

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct      120
ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctgggtc ccgtctcgtc c                                           351
  
```

<210> SEQ ID NO 141  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence

-continued

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1; VL

&lt;400&gt; SEQUENCE: 141

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1                   5                   10                   15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser  
           20                   25                   30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
           35                   40                   45  
 Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
           50                   55                   60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65                   70                   75                   80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro  
           85                   90                   95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
           100                   105

&lt;210&gt; SEQ ID NO 142

&lt;211&gt; LENGTH: 324

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1; VL

&lt;400&gt; SEQUENCE: 142

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc   60  
 ctctcttgca gggccagtc gagtgtagc cgcagctact tagcctggta ccagcagaaa   120  
 cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggcatccca   180  
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag   240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattcccc tacgttcggc   300  
 caggggacca aagtggaaat caaa   324

&lt;210&gt; SEQ ID NO 143

&lt;211&gt; LENGTH: 116

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1; VH

&lt;400&gt; SEQUENCE: 143

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1                   5                   10                   15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His  
           20                   25                   30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
           35                   40                   45  
 Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys  
           50                   55                   60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
 65                   70                   75                   80  
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
           85                   90                   95  
 Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
           100                   105                   110



-continued

<210> SEQ ID NO 147  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 22A3; VH

<400> SEQUENCE: 147

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45
Ser Ala Ile Ile Gly Ser Gly Ser Ile Thr Tyr Tyr Ala Asp Ser Val
          50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
          65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85          90          95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
          100          105          110
Val Thr Val Ser Ser
          115

```

<210> SEQ ID NO 148  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 22A3; VH

<400> SEQUENCE: 148

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attattggta gtggtagtat cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctggtea ccgtctcgag t              351

```

<210> SEQ ID NO 149  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 29B11; VL

<400> SEQUENCE: 149

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1          5          10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
          20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
          35          40          45
Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
          50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
          65          70          75          80

```

-continued

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
                   85                                  90                                  95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
                   100                                  105

<210> SEQ ID NO 150  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 29B11; VL

<400> SEQUENCE: 150

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60  
 ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa 120  
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180  
 gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240  
 cctgaagatt ttgcagtgt ttactgtcag cagggtatta tgettccccc gacgttcggc 300  
 caggggacca aagtggaaat caaa 324

<210> SEQ ID NO 151  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 29B11; VH

<400> SEQUENCE: 151

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1                  5                                  10                                  15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
                   20                                  25                                  30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
                   35                                  40                                  45  
 Ser Ala Ile Ile Gly Ser Gly Gly Ile Thr Tyr Tyr Ala Asp Ser Val  
                   50                                  55                                  60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65                  70                                  75                                  80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
                   85                                  90                                  95  
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu  
                   100                                  105                                  110  
 Val Thr Val Ser Ser  
                   115

<210> SEQ ID NO 152  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 29B11; VH

<400> SEQUENCE: 152

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60  
 tcctgtgcag cctccggatt caccttagc agttatgcta tgagctgggt ccgccaggct 120  
 ccaggggaagg ggctggagtg ggtctcagct attattgcta gtggtgggat cacatactac 180

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```
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttggtggtt ttaactactg gggccaagga accctgggtc ccgtctcgag t 351
```

```
<210> SEQ ID NO 153
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 23C10; VL
```

```
<400> SEQUENCE: 153
```

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35          40          45
Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100         105
```

```
<210> SEQ ID NO 154
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 23C10; VL
```

```
<400> SEQUENCE: 154
```

```
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtc gagtgtagc cgcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggaatccca 180
gacagggtca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattcccc tacgttcggc 300
caggggacca aagtggaaat caaa 324
```

```
<210> SEQ ID NO 155
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 23C10; VH
```

```
<400> SEQUENCE: 155
```

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ser
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Thr Asn Gly Asn Tyr Thr Tyr Tyr Ala Asp Ser Val
```

-continued

---

50	55	60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
65	70	75	80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu			
	100	105	110
Val Thr Val Ser Ser			
	115		

<210> SEQ ID NO 156  
 <211> LENGTH: 351  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 23C10; VH  
  
 <400> SEQUENCE: 156

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccgatt caccttagc agttctgcc tgagctgggt ccgccaggct	120
ccaggaagg ggtggagtg ggtctcagct attagtacta atggttaatta tacatactac	180
gcagactccg tgaagggcc gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg	300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t	351

<210> SEQ ID NO 157  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_C3B6; VL  
  
 <400> SEQUENCE: 157

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly	
1	15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp	
20	30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile	
35	45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly	
50	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro	
65	80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala	
85	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
100	105

<210> SEQ ID NO 158  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_C3B6; VL  
  
 <400> SEQUENCE: 158

gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc	60
--	----

-continued

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```

atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgctt gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
agggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgacg aatggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

```

```

<210> SEQ ID NO 159
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_C3B6; VH

```

```

<400> SEQUENCE: 159

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20     25     30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35     40     45
Gly Ala Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50     55     60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65     70     75     80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85     90     95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100    105    110
Gln Gly Thr Thr Val Thr Val Ser Ser
115    120

```

```

<210> SEQ ID NO 160
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_C3B6; VH

```

```

<400> SEQUENCE: 160

```

```

cagggtcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcgaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggagct atcatcccga tccttggtat cgaaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

```

```

<210> SEQ ID NO 161
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_6A12; VL

```

```

<400> SEQUENCE: 161

```

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

```

-continued

---

1	5	10	15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp			
	20	25	30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile			
	35	40	45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly			
	50	55	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro			
	65	70	75
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala			
	85	90	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys			
	100	105	

<210> SEQ ID NO 162  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6A12; VL

<400> SEQUENCE: 162

gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc	60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca	120
gggaaagccc ctaagcgct gatctatgct gcacccagtt tgcagagtgg cgtcccatca	180
aggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct	240
gaagattttg ccacctatta ctgcttgag aatggtctgc agcccgcgac gtttgccag	300
ggcaccaaag tcgagatcaa g	321

<210> SEQ ID NO 163  
 <211> LENGTH: 121  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6A12; VH

<400> SEQUENCE: 163

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser			
1	5	10	15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr			
	20	25	30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met			
	35	40	45
Gly Val Ile Ile Pro Ile Leu Gly Thr Ala Asn Tyr Ala Gln Lys Phe			
	50	55	60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr			
	65	70	75
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly			
	100	105	110
Gln Gly Thr Thr Val Thr Val Ser Ser			
	115	120	

<210> SEQ ID NO 164  
 <211> LENGTH: 363

-continued

<212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6A12; VH

<400> SEQUENCE: 164

```

caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcctgcaagg cctccggagg cacattcagc agctatgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggagtg atcatcccta tccttggtac cgcaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacgggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tca

```

<210> SEQ ID NO 165  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_C3A6; VL

<400> SEQUENCE: 165

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1             5             10             15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
20            25            30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35            40            45
Tyr Asp Ser Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50            55            60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65            70            75            80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85            90            95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100           105

```

<210> SEQ ID NO 166  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_C3A6; VL

<400> SEQUENCE: 166

```

gacatccaga tgaccacgac tccttcctcc ctgtctgcat ctgtcggaga ccggggtcacc      60
atcacctgcc gggcaagtca gggcattcgt aatgttttag gctgggtacca gcagaagcca      120
gggaaagccc ctaagcgect gatctatgat tcgtccagtt tgcagagtgg cgteccatca      180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct      240
gaagattttg ccacctatta ctgcttgagc aatggtctgc agcccgcgac gtttggccag      300
ggcaccaaag tcgagatcaa g

```

<210> SEQ ID NO 167  
 <211> LENGTH: 121  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence

-continued

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10\_C3A6; VH

&lt;400&gt; SEQUENCE: 167

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser Ser  
 115 120

&lt;210&gt; SEQ ID NO 168

&lt;211&gt; LENGTH: 363

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10\_C3A6; VH

&lt;400&gt; SEQUENCE: 168

cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60  
 tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120  
 cctggacaag ggctgcagtg gatgggaggg atcatcccta tctttggtac agcaaaactac 180  
 gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240  
 atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300  
 ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360  
 tca 363

&lt;210&gt; SEQ ID NO 169

&lt;211&gt; LENGTH: 107

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10\_D1A2\_wt; VL

&lt;400&gt; SEQUENCE: 169

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val  
 20 25 30  
 Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile  
 35 40 45  
 Tyr Asp Ala Tyr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
 Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala

-continued

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85	90	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys		
100	105	

<210> SEQ ID NO 170  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_D1A2\_wt; VL

<400> SEQUENCE: 170

gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtcggaga ccggggtcacc	60
atcacctgcc gggcaagtca ggggattcgt aatgttttag gctggtacca gcagaagcca	120
gggaaagccc ctaagcgct gatctatgat gcttacagct tgcagagtgg cgtcccatca	180
aggttcagcg gcggtggatc cgggacagag ttactctca ccatcagcag cttgcagcct	240
gaagattttg ccacctatta ctgcttgag aatggtctgc agcccgcgac gtttgccag	300
ggcaccaaag tcgagatcaa g	321

<210> SEQ ID NO 171  
 <211> LENGTH: 121  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_D1A2\_wt; VH

<400> SEQUENCE: 171

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser	
1 5 10 15	
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr	
20 25 30	
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met	
35 40 45	
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe	
50 55 60	
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr	
65 70 75 80	
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly	
100 105 110	
Gln Gly Thr Thr Val Thr Val Ser Ser	
115 120	

<210> SEQ ID NO 172  
 <211> LENGTH: 363  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_D1A2\_wt; VH

<400> SEQUENCE: 172

cagggtcaat tgggtcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc	60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc	120
cctggacaag ggctcgagt gatgggagg atcatcccta tctttggtac agcaaactac	180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac	240

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```

atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

```

```

<210> SEQ ID NO 173
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_D1A2_VD; VL

```

```

<400> SEQUENCE: 173

```

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1         5         10        15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20        25        30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35        40        45
Tyr Asp Ala Tyr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50        55        60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65        70        75        80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85        90        95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100       105

```

```

<210> SEQ ID NO 174
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_D1A2_VD; VL

```

```

<400> SEQUENCE: 174

```

```

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccggggtcacc 60
atcacctgcc gggcaagtca ggggattcgt aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgctt gatctatgat gcttacagct tgcagagtgg cgtcccatca 180
agggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgagc aatggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

```

```

<210> SEQ ID NO 175
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_D1A2_VD; VH

```

```

<400> SEQUENCE: 175

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1         5         10        15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20        25        30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35        40        45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe

```

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50	55	60	
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr			
65	70	75	80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly			
	100	105	110
Gln Gly Thr Thr Val Thr Val Ser Ser			
	115	120	

<210> SEQ ID NO 176  
 <211> LENGTH: 363  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_D1A2\_VD; VH  
  
 <400> SEQUENCE: 176

cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc	60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc	120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac	180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac	240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac	300
ggttacgctt actacgggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc	360
tca	363

<210> SEQ ID NO 177  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_O7D8; VL  
  
 <400> SEQUENCE: 177

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly	
1	15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Arg Asn Val	
20	30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile	
35	45
Tyr Asp Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly	
50	60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro	
65	80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala	
85	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
100	105

<210> SEQ ID NO 178  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_O7D8; VL  
  
 <400> SEQUENCE: 178

-continued

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```

gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc      60
atcacctgcc gggcaagtca gagcattcgt aatgttttag gctggtacca gcagaagcca      120
gggaaagccc ctaagcgctt gatctatgat gtgtccagtt tgcagagtgg cgtcccatca      180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct      240
gaagattttg ccacctatta ctgcttgagc aatgggtctgc agcccgcgac gtttggccag      300
ggcaccaaag tcgagatcaa g                                           321

```

```

<210> SEQ ID NO 179
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_07D8; VH

```

```

<400> SEQUENCE: 179

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
          20          25          30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
          35          40          45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
          50          55          60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
          65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
          85          90          95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
          100          105          110
Gln Gly Thr Thr Val Thr Val Ser Ser
          115          120

```

```

<210> SEQ ID NO 180
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_07D8; VH

```

```

<400> SEQUENCE: 180

```

```

caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcttgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tca                                           363

```

```

<210> SEQ ID NO 181
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 2B10_01F7; VL

```

```

<400> SEQUENCE: 181

```

-continued

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val  
 20 25 30  
 Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile  
 35 40 45  
 Tyr Asp Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
 Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala  
 85 90 95  
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> SEQ ID NO 182  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_O1F7; VL

<400> SEQUENCE: 182

gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccggggtcacc 60  
 atcacctgcc gggcaagtca gggcattcgt aatgttttag gctggtacca gcagaagcca 120  
 gggaaagccc ctaagcgct gatctatgat gcgtccagtt tgcagagtgg cgtcccatca 180  
 aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240  
 gaagattttg ccacctatta ctgcctgcag aatggtctgc agccgcgcac gtttgccag 300  
 ggcaccaaag tcgagatcaa g 321

<210> SEQ ID NO 183  
 <211> LENGTH: 121  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_O1F7; VH

<400> SEQUENCE: 183

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
 20 25 30  
 Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser  
 115 120

-continued

<210> SEQ ID NO 184  
 <211> LENGTH: 363  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_01F7; VH

<400> SEQUENCE: 184

```
cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacgggtgc ttttgactac tggggccaag ggaccacggt gaccgtctcc      360
tca                                                                                   363
```

<210> SEQ ID NO 185  
 <211> LENGTH: 107  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6H10; VL

<400> SEQUENCE: 185

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1             5             10             15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
20            25            30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35            40            45
Gln Ala Ala Thr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50            55            60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65            70            75            80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85            90            95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100           105
```

<210> SEQ ID NO 186  
 <211> LENGTH: 321  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6H10; VL

<400> SEQUENCE: 186

```
gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc      60
atcacctgcc gggcaagtca gggcattcgt aatgttttag gctgggtacca gcagaagcca      120
gggaaagccc ctaagcgct gatccaggct gctaccagtt tgcagagtgg cgtcccatca      180
agggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct      240
gaagattttg ccacctatta ctgcttgagc aatggtctgc agcccgcgac gtttgccag      300
ggcaccaaag tcgagatcaa g                                                                                   321
```

<210> SEQ ID NO 187  
 <211> LENGTH: 121

-continued

<212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6H10; VH

<400> SEQUENCE: 187

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1           5           10           15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
          20           25           30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
          35           40           45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
          50           55           60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
          65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
          85           90           95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
          100          105          110
Gln Gly Thr Thr Val Thr Val Ser Ser
          115          120
```

<210> SEQ ID NO 188  
 <211> LENGTH: 363  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10\_6H10; VH

<400> SEQUENCE: 188

```
cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tctctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tca                                                    363
```

<210> SEQ ID NO 189  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A; VL

<400> SEQUENCE: 189

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
          20           25           30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
          35           40           45
Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
          65           70           75           80
```

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Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu  
85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> SEQ ID NO 190

<211> LENGTH: 324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CH1A1A; VL

<400> SEQUENCE: 190

gataccaga tgaccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc 60  
atcacttgca aggccagtgc ggctgtgggt acgtatgttg cgtgggtatca gcagaaacca 120  
gggaaagcac ctaagctcct gatctattcg gcacccctacc gcaaaagggg agtcccatca 180  
agggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240  
gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cactgttggc 300  
cagggcacca agctcgagat caag 324

<210> SEQ ID NO 191

<211> LENGTH: 121

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CH1A1A; VH

<400> SEQUENCE: 191

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe  
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly  
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> SEQ ID NO 192

<211> LENGTH: 363

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CH1A1A; VH

<400> SEQUENCE: 192

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg 60  
tcctgcaagg ccagcggtcta caccttcacc gagttcgga tgaactgggt ccgacaggct 120  
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180

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```

gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac   240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac   300
ttcgctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct   360
agc                                                                    363

```

```

<210> SEQ ID NO 193
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 28H1 Fab HC-Fc hole (LALA P329G)

```

```

<400> SEQUENCE: 193

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50          55          60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65          70          75          80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85          90          95
Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100         105         110
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115         120         125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130         135         140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145         150         155         160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165         170         175
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180         185         190
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195         200         205
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210         215         220
Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
225         230         235         240
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245         250         255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260         265         270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275         280         285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290         295         300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305         310         315         320
Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser

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325					330					335				
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu	Pro
			340					345					350	
Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Ala
		355					360					365		Val
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380			Gly
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385				390					395					400
Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
			405					410						415
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
		420					425						430	His
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
		435					440						445	

&lt;210&gt; SEQ ID NO 194

&lt;211&gt; LENGTH: 1338

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 194

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gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg      60
tcctgcgcgc cctcgcgctt caccttctcc tcccacgcc tgcctctggg cgcacaggct      120
cctggcaaa gcttggaatg ggtgtccgcc atctgggcct cggcgcagca gtactacgcc      180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg      240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgcca gggctggctg      300
ggcaacttgc actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag      360
ggccctccgc tgttccccct ggcccccagc agcaagagca ccagcggcgg cacagccgct      420
ctgggctgcc tggteaagga ctacttcccc gagcccgtga ccgtgtcctg gaacagcgga      480
gccctgacct cggcgtgca caccttcccc gccgtgtgc agagttctgg cctgtatagc      540
ctgagcagcg tggtcaccgt gccttctagc agcctgggca ccagaccta catctgcaac      600
gtgaaccaca agcccagcaa caccaagggt gacaagaagg tggagcccaa gagctgcgac      660
aaaactcaca catgcccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcca gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggtctcca acaaaacct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg      1020
cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac      1080
caggtcagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatgc cgtggagtgg      1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac      1200
ggctccttct tcctcgtgag caagctcacc gtggacaaga gcaggtggca gcaggggaac      1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc      1320
tccctgtctc cgggtaaa

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<210> SEQ ID NO 195
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> SEQUENCE: 195

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50          55          60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65          70          75          80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85          90          95
Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100         105        110
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115        120        125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130        135        140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145        150        155        160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165        170        175
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180        185        190
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195        200        205
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210        215        220
Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
225        230        235        240
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245        250        255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260        265        270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275        280        285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290        295        300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305        310        315        320
Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
325        330        335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340        345        350
Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
355        360        365

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Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 405 410 415  
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 420 425 430  
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly  
 435 440 445  
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser Ser  
 450 455 460  
 Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu  
 465 470 475 480  
 Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr  
 485 490 495  
 Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu  
 500 505 510  
 Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val  
 515 520 525  
 Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu  
 530 535 540  
 Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr  
 545 550 555 560  
 Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe  
 565 570 575  
 Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr  
 580 585 590

&lt;210&gt; SEQ ID NO 196

&lt;211&gt; LENGTH: 1776

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 196

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gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg      60
tcctgcgcgc cctccggett caccttctcc tccaacgcca tgtcctgggt ccgacaggct      120
cctggc aaag gcctggaatg ggtgtccgcc atctgggcct ccggcgagca gtactacgcc      180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg      240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgcca gggctggctg      300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag      360
ggcccatcgg tcttccccct ggcacctctc tccaagagca cctctggggg cacagcggcc      420
ctgggctgcc tggtaagga ctacttcccc gaaccggtga cgggtgtcgtg gaactcaggc      480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcagg actctactcc      540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca ccagaccta catctgcaac      600
gtgaatcaca agcccagcaa caccaagggtg gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgccacc gtgccagca cctgaagctg cagggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacacctc atgatctccc ggaccttgga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagacct gaggtcaagt tcaactggta cgtggacggc      840

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gtggagggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt    900
gtggtcagcg tcctcaccgt cctgcaccag gactgggtga atggcaagga gtacaagtgc    960
aagggtctcca acaaagccct cgcgccccc atcgagaaaa ccatctccaa agccaaaggg   1020
cagccccgag aaccacaggt gtacacctg ccccatgcc gggatgagct gaccaagaac   1080
caggtcagcc tgtggtgctt ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg   1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac   1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcagggggaa   1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc   1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct   1380
cctgcctcct ccagcaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg   1440
cagatgatcc tgaacggcat caacaactac aagaacccca agctgaccgc gatgctgacc   1500
gccaagttcg ccatgcccaa gaaggccacc gagctgaaac atctgcagtg cctggaagag   1560
gaactgaagc ctctggaaga ggtgctgaac ggcgcccagt ccaagaactt ccacctgagg   1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca   1680
accttcatgt gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccgggtg   1740
atcaccttcg ccagttccat catctccacc ctgacc                                1776

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<210> SEQ ID NO 197
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 wt
<400> SEQUENCE: 197

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50          55          60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65          70          75          80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85          90          95
Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100         105         110
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115         120         125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130         135         140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145         150         155         160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165         170         175
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180         185         190

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Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr
		195					200					205			
Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr
	210					215					220				
Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val	Phe
225					230					235					240
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
				245					250					255	
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val
			260					265					270		
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr
		275					280					285			
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
	290					295					300				
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys
305					310					315					320
Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser
				325					330					335	
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro
			340					345					350		
Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu	Val
		355					360					365			
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly
	370					375					380				
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp
385					390					395					400
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp
				405					410					415	
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His
			420					425					430		
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly
		435					440					445			
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Pro	Thr	Ser	Ser
	450					455					460				
Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	Leu
465					470					475					480
Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	Thr
				485					490					495	
Arg	Met	Leu	Thr	Phe	Lys	Phe	Tyr	Met	Pro	Lys	Lys	Ala	Thr	Glu	Leu
			500					505					510		
Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val
		515					520					525			
Leu	Asn	Leu	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	Leu
	530					535					540				
Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	Thr
545					550					555					560
Thr	Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr	Ile	Val	Glu	Phe
				565					570					575	
Leu	Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser	Ile	Ile	Ser	Thr	Leu	Thr
			580					585					590		

&lt;210&gt; SEQ ID NO 198

&lt;211&gt; LENGTH: 1776

&lt;212&gt; TYPE: DNA

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&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 wt

&lt;400&gt; SEQUENCE: 198

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gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg    60
tcttgcgccg cctcgggctt caccttctcc tcccacgcca tgtcctgggt ccgacaggct    120
cctggcaaa gcttggaatg ggtgtccgcc atctgggcct ccggcgagca gtactacgcc    180
gactctgtga agggccgggt caccatctcc cgggacaact ccaagaacac cctgtacctg    240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgcca gggtggctg    300
ggcaacttcg actactgggg acagggcacc ctggtcacgg tgtccagcgc tagcaccaag    360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc    420
ctgggctgcc tggtaagga ctacttcccc gaaccgggta cgggtgtcgtg gaactcaggc    480
gccctgacca gggcggtgca caccttcccg gctgtctac agtcctcagg actctactcc    540
ctcagcagcg tggtagccgt gccctccagc agcttgggca cccagaccta catctgcaac    600
gtgaatcaca agcccagcaa caccaagggt gacaagaaag ttgagcccaa atcttgtgac    660
aaaactcaca catgcccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc    720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc    780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc    840
gtggagggtg ataatgcaa gacaaagccg cgggaggagc agtacaacag cactgacctg    900
gtggtcagcg tctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc    960
aaggtctcca acaaaagcct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg    1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac    1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg    1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac    1200
ggctccttct tctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaac    1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc    1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct    1380
cctacatcct ccagcaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg    1440
cagatgatcc tgaacggcat caacaactac aagaacccca agctgaccgc gatgctgacc    1500
ttcaagttct acatgcccaa gaaggccacc gagctgaaac atctgcagtg cctggaagag    1560
gaactgaagc ctctggaaga ggtgctgaac ctggcccagt ccaagaactt ccacctgagg    1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca    1680
accttcattg gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccgggtg    1740
atcaccttcg ccagttccat catctccacc ctgacc                                1776

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&lt;210&gt; SEQ ID NO 199

&lt;211&gt; LENGTH: 574

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-15  
(E53A N79A)

&lt;400&gt; SEQUENCE: 199

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys  
 50 55 60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
 65 70 75 80  
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95  
 Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100 105 110  
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125  
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140  
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160  
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175  
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190  
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350  
 Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val  
 355 360 365  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 405 410 415  
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 420 425 430

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Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Gly	Gly
		435					440					445			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Asn	Trp	Val	Asn
	450					455					460				
Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln	Ser	Met	His
465				470						475					480
Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro	Ser	Cys	Lys
			485						490					495	
Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val	Ile	Ser	Leu
			500					505					510		
Ala	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn	Leu	Ile	Ile
		515					520					525			
Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Ala	Val	Thr	Glu	Ser	Gly
	530					535					540				
Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys	Glu	Phe	Leu
545				550						555					560
Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr	Ser		
			565						570						

<210> SEQ ID NO 200  
 <211> LENGTH: 1722  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-15  
 (E53A N79A)

<400> SEQUENCE: 200

gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg	60
tcctcgcccg cctccggctt cacccttctc tcccacgcca tgtcctgggt ccgacaggct	120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct ccgcgagca gtactacgcc	180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg	240
cagatgaact cctgcgggc cgaggacacc gccgtgtact actgtgccaa gggctggctg	300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag	360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc	420
ctgggctgcc tggtaagga ctacttcccc gaaccgggtg cggtgtcgtg gaactcaggc	480
gccctgacca gcgcggtgca caccctcccg gctgtcctac agtcctcagg actctactcc	540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca ccagaccta catctgcaac	600
gtgaatcaca agcccagcaa caccaagggt gacaagaaag ttgagcccaa atcttgtgac	660
aaaactcaca catgccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc	720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc	780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc	840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt	900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc	960
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg	1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac	1080
caggctagcc tgtggtgctt ggtcaaaggc ttctatccca gcgacatgc cgtggagtgg	1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac	1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac	1260

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gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1320
tccctgtctc cgggttccgg cggcggaggc tccggaggcg gaggttcttg cgaggtggc 1380
aactgggtga atgtaataag tgatttgaag aaaattgaag atcttattca atctatgcat 1440
attgatgcta ctttatatac ggaaagtgat gttcacccca gttgcaaagt aacagcaatg 1500
aagtgccttc tcttgaggtt acaagttatt tcacttgcgt ccggagatgc aagtattcat 1560
gatacagtag aaaatctgat catcctagca aacaacagtt tgtcttctaa tggggctgta 1620
acagaatctg gatgcaaaga atgtgaggaa ctggaggaaa aaaatattaa agaatttttg 1680
cagagttttg tacatattgt ccaaatgttc atcaacactt ct 1722

```

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<210> SEQ ID NO 201
<211> LENGTH: 447
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)

```

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<400> SEQUENCE: 201

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20         25         30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35         40         45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50         55         60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65         70         75         80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85         90         95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100        105        110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115        120        125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130        135        140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145        150        155        160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165        170        175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180        185        190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195        200        205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210        215        220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225        230        235        240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245        250        255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260        265        270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys

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275	280	285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser 290 295 300		
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys 305 310 315 320		
Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile 325 330 335		
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro 340 345 350		
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala 355 360 365		
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn 370 375 380		
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser 385 390 395 400		
Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg 405 410 415		
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu 420 425 430		
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 435 440 445		

&lt;210&gt; SEQ ID NO 202

&lt;211&gt; LENGTH: 1341

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 202

```

gagggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgccg tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
ctgggtaatt ttgactactg gggccaagga accctgggtc cgtctcagag tgetagcacc      360
aaggggccct ccgtgttccc cctggccccc agcagcaaga gcaccagcgg cggcacagcc      420
gctctgggct gcctgggtcaa ggactacttc cccgagcccg tgaccgtgtc ctggaacagc      480
ggagccctga cctccggcgt gcacaccttc cccgccgtgc tgcagagtgc tggcctgtat      540
agcctgagca gcgtgggtcac cgtgccttct agcagcctgg gcaccagac ctacatctgc      600
aacgtgaacc acaagcccag caacaccaag gtggacaaga aggtggagcc caagagctgc      660
gacaaaaactc acacatgccc accgtgcccc gcacctgaag ctgcaggggg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      780
tgcggtggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac      900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaagggtc ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag     1080
aaccaggtca gcctctcgtg cgcagtcaaa ggcttctatc ccagcgacat cgccgtggag     1140

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tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccggt gctggactcc 1200
gacggctcct tcttctcctg gagcaagctc accgtggaca agagcaggtg gcagcagggg 1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
ctctccctgt ctccgggtaa a 1341
```

```
<210> SEQ ID NO 203
<211> LENGTH: 593
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4G8 Fab HC-Fc knob (LALA P329G)-IL-2 qm
```

```
<400> SEQUENCE: 203
```

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115         120         125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130         135         140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145         150         155         160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165         170         175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180         185         190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195         200         205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210         215         220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225         230         235         240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245         250         255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260         265         270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275         280         285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290         295         300
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305         310         315         320
```

Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile
				325					330					335	
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
			340					345					350		
Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu
		355					360					365			
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380				
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385					390					395					400
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
				405					410					415	
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
			420					425					430		
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly
		435					440					445			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Pro	Ala	Ser
	450					455					460				
Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp
465					470					475					480
Leu	Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu
				485					490					495	
Thr	Arg	Met	Leu	Thr	Ala	Lys	Phe	Ala	Met	Pro	Lys	Lys	Ala	Thr	Glu
			500					505					510		
Leu	Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu
		515					520					525			
Val	Leu	Asn	Gly	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp
	530					535					540				
Leu	Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu
545					550					555					560
Thr	Thr	Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr	Ile	Val	Glu
				565					570					575	
Phe	Leu	Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser	Ile	Ile	Ser	Thr	Leu
			580					585					590		

```
<210> SEQ ID NO 204
<211> LENGTH: 1779
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4G8 Fab HC-Fc knob (LALA P329G)-IL-2 qm
```

gaggtgcaat tgttggaagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc	60
tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct	120
ccagggaagg ggtctggagt ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggtgg	300
ctgggtaatt ttgactactg gggccaagga accctggtca ccgtctcgag tgctagcacc	360
aaggggcccat cggctctccc cctggcacc cctctcaaga gcacctctgg gggcacagcg	420
gccctgggct gctcgtcaa ggactacttc cccgaaccgg tgacgggtgc gtggaactca	480

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ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtccctc aggactctac 540
tccctcagca gcgtagtgac cgtgcctccc agcagcttgg gcacccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaaatc acacatgccc accgtgccc gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaacctctc caaagccaaa 1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag 1080
aaccaggtca gcctgtggtg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1140
tgaggagagca atgggcagcc ggagaacaac tacaagacca cgctcccggt gctggactcc 1200
gacggctcct tcttctctca cagcaagctc accgtggaca agagcaggtg gcagcagggg 1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggttcttg cgaggtggc 1380
gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat 1440
ctgcagatga tcctgaacgg catcaacaac tacaagaacc ccaagctgac ccggtgctg 1500
accgccaagt tcgcatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa 1560
gaggaaactga agcctctgga agaggtgctg aacggcgccc agtccaagaa ctccacctg 1620
aggcctcggg acctgatctc caacatcaac gtgatcgtgc tggaactgaa gggctccgag 1680
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg 1740
tggatcacct tcgcccagtc catcatctcc accctgacc 1779

```

&lt;210&gt; SEQ ID NO 205

&lt;211&gt; LENGTH: 215

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4G8 Fab LC

&lt;400&gt; SEQUENCE: 205

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1             5             10             15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
20            25            30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35            40            45

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50            55            60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65            70            75            80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85            90            95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100           105           110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115           120           125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu

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130	135	140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser		
145	150	155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu		
	165	170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val		
	180	185 190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys		
	195	200 205
Ser Phe Asn Arg Gly Glu Cys		
	210	215

&lt;210&gt; SEQ ID NO 206

&lt;211&gt; LENGTH: 645

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4G8 Fab LC

&lt;400&gt; SEQUENCE: 206

```

gagatcgtgc tgacccagtc ccccggcacc ctgtctctga gccctggcga gagagccacc      60
ctgtcctgca gagcctccca gtcctgtgcc cggtcctacc tcgcctggta tcagcagaag      120
cccggccagg cccctcgget gctgatcacc ggcgccteta ccagagccac cggcatecct      180
gaccggttct ccggtctctgg ctccggcacc gacttcaccc tgaccatctc ccggctggaa      240
cccgaggact tcgccgtgta ctactgccag cagggccagg tcatccctcc cacctttggc      300
cagggcacca aggtggaaat caagcgtacg gtggctgcac catctgtctt catcttcccg      360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgctgtgt gaataacttc      420
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc      480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcacccctg      540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag      600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgtt      645

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&lt;210&gt; SEQ ID NO 207

&lt;211&gt; LENGTH: 447

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 207

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	
20 25 30	
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val	
50 55 60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	
65 70 75 80	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu	
100 105 110	

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Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu  
 115 120 125  
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys  
 130 135 140  
 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser  
 145 150 155 160  
 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser  
 165 170 175  
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser  
 180 185 190  
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn  
 195 200 205  
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His  
 210 215 220  
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val  
 225 230 235 240  
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr  
 245 250 255  
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu  
 260 265 270  
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys  
 275 280 285  
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser  
 290 295 300  
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys  
 305 310 315 320  
 Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile  
 325 330 335  
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro  
 340 345 350  
 Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala  
 355 360 365  
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
 370 375 380  
 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser  
 385 390 395 400  
 Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg  
 405 410 415  
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu  
 420 425 430  
 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 435 440 445

&lt;210&gt; SEQ ID NO 208

&lt;211&gt; LENGTH: 1341

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 208

gaggtgcagc tgctcgaaag cggcgaggga ctggtgcagc ctggcggcag cctgagactg 60

tcttgccgag ccagcggcgt caccttcagc agctacgcca tgagctgggt ccgccaggcc 120

cctggcaagg gactggaatg ggtgtccgcc atcatcggt ctggcgccag cacctactac 180

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gccgacagcg tgaagggcgc gttcaccatc agccgggaca acagcaagaa caccctgtac   240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggatgg   300
ttcggcggct tcaactactg gggacagggc accctggtea cagtgtccag cgctagcacc   360
aagggccctt ccgtgttccc cctggccccc agcagcaaga gcaccagcgg cggcacagcc   420
gctctgggct gcctggtea ggactacttc cccgagcccg tgaccgtgtc ctggaacagc   480
ggagccctga cctcggcgt gcacaccttc cccgcgtgc tgcagagttc tggcctgtat   540
agcctgagca gcgtggtea cgtgccttct agcagcctgg gcaccagac ctacatctgc   600
aacgtgaacc acaagcccag caacaccaag gtggacaaga aggtggagcc caagagctgc   660
gacaaaactc acacatgccc accgtgccc gcacctgaag ctgcaggggg accgtcagtc   720
ttcctcttcc ccccaaaacc caaggacacc ctcctgatct cccggacccc tgaggtcaca   780
tgcgtggtgg tggacgtgag ccacgaagac cctgaggtea agttcaactg gtacgtggac   840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac   900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag   960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaacctctc caaagccaaa  1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag  1080
aaccaggtea gcctctcgtg cgcagtcaaa ggcttctatc ccagcgacat cgcctgggag  1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccg tctggactcc  1200
gacggctcct tcttcctcgt gagcaagctc accgtggaca agagcagggtg gcagcagggg  1260
aacgtcttct catgctcgtg gatgcatgag gctctgcaca accactacac gcagaagagc  1320
ctctccctgt ctccgggtaa a                                     1341

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&lt;210&gt; SEQ ID NO 209

&lt;211&gt; LENGTH: 593

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 209

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115         120         125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130         135         140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser

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145	150	155	160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser			
	165	170	175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser			
	180	185	190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn			
	195	200	205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His			
	210	215	220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val			
	225	230	235
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr			
	245	250	255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu			
	260	265	270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys			
	275	280	285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser			
	290	295	300
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys			
	305	310	315
Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile			
	325	330	335
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro			
	340	345	350
Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu			
	355	360	365
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn			
	370	375	380
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser			
	385	390	395
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg			
	405	410	415
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu			
	420	425	430
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly			
	435	440	445
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser			
	450	455	460
Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp			
	465	470	475
Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu			
	485	490	495
Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu			
	500	505	510
Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu			
	515	520	525
Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp			
	530	535	540
Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu			
	545	550	555
Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu			
	565	570	575

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Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu  
 580 585 590

Thr

<210> SEQ ID NO 210  
 <211> LENGTH: 1779  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> SEQUENCE: 210

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gaggtgcagc tgctcgaaag cgccggagga ctggtgcagc ctggcggcag cctgagactg    60
tcttgcgccg ccagcggett caccttcagc agctacgcca tgagctgggt ccgccaggcc    120
cctggcaagg gactggaatg ggtgtccgcc atcatcggtt ctggcgccag cacctactac    180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac    240
ctgcagatga acagcctgcg ggccgaggac accgcccgtt actactgcgc caagggatgg    300
ttcgcggtct tcaactactg gggacagggc accctgggtc cagtgtccag cgctagcacc    360
aaggggcccat cggtcttccc cctggcacc cctccaaga gcacctctgg gggcacagcg    420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca    480
ggcgccctga ccagcggcgt gcacaccttc ccggctgttc tacagtcttc aggactctac    540
tccctcagca gcgtgggtgac cgtgcccttc agcagcttgg gcaccagac ctacatctgc    600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt    660
gacaaaactc acacatgccc accgtgccc gacacctgaag ctgcaggggg accgtcagtc    720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca    780
tgcggtggtg tggacgtgag ccacgaagac cctgagggtc agttcaactg gtacgtggac    840
ggcggtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac    900
cgtgtgggtc gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag    960
tgcaaggtct ccaacaaagc cctcgcgccc cccatcgaga aaacctctc caaagccaaa   1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag   1080
aaccagggtc gcctgtggtg cctgggtcaa ggcttctatc ccagcgacat cgcctgggag   1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctccgt gctggactcc   1200
gacggctcct tcttctctca cagcaagtc accgtggaca agagcagggt gcagcagggg   1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc   1320
ctctccctgt ctccgggtgg cgccggaggc tccggaggcg gaggttctgg cggaggtggc   1380
gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat   1440
ctgcagatga tcttgaacgg catcaacaac tacaagaacc ccaagctgac ccggatgctg   1500
accgccaagt tcgccatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa   1560
gaggaaactg agcctctgga agaggtgctg aacggcgccc agtccaagaa cttccacctg   1620
aggcctcggg acctgatctc caacatcaac gtgatcgtc tggaactgaa gggctccgag   1680
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg   1740
tggatcacct tcgccagtc catcatctcc accctgacc                               1779

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<210> SEQ ID NO 211  
 <211> LENGTH: 215

-continued

<212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 3F2 Fab LC

<400> SEQUENCE: 211

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser  
 20 25 30  
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro  
 85 90 95  
 Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala  
 100 105 110  
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125  
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140  
 Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
 145 150 155 160  
 Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 165 170 175  
 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 180 185 190  
 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
 195 200 205  
 Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> SEQ ID NO 212  
 <211> LENGTH: 645  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 3F2 Fab LC

<400> SEQUENCE: 212

gagatcgtgc tgaccagtc ccccggcacc ctgtctctga gccctggcga gagagccacc 60  
 ctgtcctgca gaggctccca gtccgtgacc tctcctacc tcgcctggta tcagcagaag 120  
 cccggccagg cccctcggt gctgatcaac gtgggcagtc ggagagccac cggcacccct 180  
 gaccggttct ccggtctctg ctccggcacc gacttcaccc tgaccatctc ccggctggaa 240  
 cccgaggact tcgccgtgta ctactgccag cagggcacat tgctgcccc cacctttggc 300  
 cagggcacca aggtggaat caagcgtacg gtggccgctc cctccgtgtt catcttccca 360  
 ccctccgacg agcagctgaa gtccggcacc gcctccgtcg tgtgctgct gaacaacttc 420  
 taccctccgc aggccaaagt gcagtggaag gtggacaacg cctgcagtc cggaactcc 480  
 caggaatccg tcaccgagca ggactccaag gacagcacct actccctgtc ctccaccctg 540  
 accctgtcca aggccgacta cgagaagcac aaggtgtacg cctgcgaagt gaccaccag 600

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ggcctgtcca gccccgtgac caagtccttc aaccggggcg agtgc

645

&lt;210&gt; SEQ ID NO 213

&lt;211&gt; LENGTH: 446

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: L19 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 213

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe  
 20 25 30  
 Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ser Ile Ser Gly Ser Ser Gly Thr Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Pro Phe Pro Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100 105 110  
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125  
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140  
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160  
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175  
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190  
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro  
 340 345 350  
 Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val

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355	360	365	
Lys Gly Phe Tyr Pro Ser Asp	Ile Ala Val Glu Trp	Glu Ser Asn Gly	
370	375	380	
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp			
385	390	395	400
Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp			
405	410	415	
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His			
420	425	430	
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys			
435	440	445	
<210> SEQ ID NO 214			
<211> LENGTH: 1338			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: L19 Fab HC-Fc hole (LALA P329G)			
<400> SEQUENCE: 214			
gagggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc		60	
tcctgtgcag cctctggatt cacctttagc agtttttcga tgagctgggt ccgccaggct		120	
ccagggaagg ggctggagtg ggtctcatct atttccggtg gttcgggtac cacatactac		180	
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat		240	
ctgcaaatga acagccctgag agccgaggac acggccgtat attactgtgc gaaaccgttt		300	
ccgtattttg actactgggg ccagggaacc ctggtcaccg tctcgagtgc tagcaccaag		360	
ggccctcccg tgttccccct ggcccccagc agcaagagca ccagcggcgg cacagccgct		420	
ctgggctgcc tggteaagga ctacttcccc gagcccgta ccgtgtcctg gaacagcgga		480	
gccctgacct ccggcgtgca caccttcccc gccgtgctgc agagttctgg cctgtatagc		540	
ctgagcagcg tggtcaccgt gccttctagc agcctgggca cccagaccta catctgcaac		600	
gtgaaccaca agcccagcaa caccaagggtg gacaagaagg tggagcccaa gagctgcgac		660	
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc		720	
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc		780	
gtggtggtgg acgtgagcca cgaagacct gaggtcaagt tcaactggta cgtggacggc		840	
gtggagggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt		900	
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc		960	
aaggtctcca acaaagccct cggcgccccc atcgagaaaa ccatctccaa agccaaaggg		1020	
cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac		1080	
caggtcagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatcgc cgtggagtgg		1140	
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac		1200	
ggctccttct tcctcgtgag caagctcacc gtggacaaga gcagggtggca gcagggggaa		1260	
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc		1320	
tccctgtctc cgggtaaa		1338	

<210> SEQ ID NO 215  
 <211> LENGTH: 592  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:

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<223> OTHER INFORMATION: L19 Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 215

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe  
 20 25 30  
 Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ser Ile Ser Gly Ser Ser Gly Thr Thr Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Pro Phe Pro Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100 105 110  
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125  
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140  
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160  
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175  
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190  
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350  
 Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val  
 355 360 365  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400



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cagccccgag aaccacaggt gtacacctg ccccatgcc gggatgagct gaccaagaac 1080
caggteagcc tgtggtgect ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac 1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcagggaac 1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct 1380
cctgcctcct ccagcaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg 1440
cagatgatcc tgaacggcat caacaactac aagaacccca agctgacccg gatgctgacc 1500
gccaagttcg ccatgcccaa gaaggccacc gagctgaaac atctgcagtg cctggaagag 1560
gaactgaagc ctctggaaga ggtgctgaac ggcgccagt ccaagaactt ccactgagg 1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca 1680
accttcatgt gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccggtgg 1740
atcaccttcg ccagttccat catctccacc ctgacc 1776

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<210> SEQ ID NO 217
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L19 Fab LC

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<400> SEQUENCE: 217

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Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20          25          30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35          40          45
Ile Tyr Tyr Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50          55          60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Gly Arg Ile Pro
85          90          95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100         105         110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115         120         125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130         135         140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145         150         155         160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165         170         175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180         185         190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195         200         205
Ser Phe Asn Arg Gly Glu Cys
210         215

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<210> SEQ ID NO 218  
 <211> LENGTH: 645  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: L19 Fab LC

<400> SEQUENCE: 218

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gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcctgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggt cctcatctat tatgcatcca gcagggccac tggcatccca      180
gacagggtta gtggcagtg gtctgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag cagacgggtc gtattcctcc gacgttcggc      300
caagggacca aggtggaaat caaacgtacg gtggctgcac catctgtctt catcttcccc      360
ccatctgatg agcagttgaa atctgggaact gcctctgttg tgtgcctgct gaataacttc      420
tatcccagag agggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc      480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg      540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag      600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt                        645

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<210> SEQ ID NO 219  
 <211> LENGTH: 445  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: DP47 GS Fab HC-Fc hole (LALA P329G)

<400> SEQUENCE: 219

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100         105         110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115         120         125
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130         135         140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145         150         155         160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165         170         175
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180         185         190
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys

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195	200	205
Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys 210 215 220		
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu 225 230 235 240		
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu 245 250 255		
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys 260 265 270		
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys 275 280 285		
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu 290 295 300		
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 305 310 315 320		
Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys 325 330 335		
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser 340 345 350		
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys 355 360 365		
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln 370 375 380		
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly 385 390 395 400		
Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln 405 410 415		
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn 420 425 430		
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 435 440 445		

&lt;210&gt; SEQ ID NO 220

&lt;211&gt; LENGTH: 1335

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: DP47 GS Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 220

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gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc   60
tcctgtgcag cctccggatt caccttagc agttatgcc tgagctgggt ccgccaggct   120
ccaggggaagg ggtcggagtg ggtctcagct attagtggta gtggtggtag cacatactac   180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat   240
ctgcagatga acagccctgag agccgaggac acggccgtat attactgtgc gaaaggcagc   300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc   360
ccctccgtgt tccccctggc ccccagcagc aagagcacca gggcgggcac agccgctctg   420
ggctgcctgg tcaaggacta cttccccgag cccgtgaccg tgtcctggaa cagcggagcc   480
ctgacctccg gcgtgcacac cttccccgcc gtgctgcaga gttctggcct gtatagcctg   540
agcagcgtgg tcaccgtgcc ttctagcagc ctgggcaccc agacctacat ctgcaacgtg   600
aaccacaagc ccagcaacac caaggtggac aagaaggtgg agcccaagag ctgcgacaaa   660

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actcacacat gccaccctg cccagcacct gaagctgcag ggggaccgtc agtcttcttc 720
ttccccccaa aacccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg 780
gtggtggaag tgagccaaga agaccctgag gtcaagttca actggtacgt ggacggcgtg 840
gagggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg 900
gtcagcgtec tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcaag 960
gtctccaaca aagccctcgg cgtccctcgc gagaaaacca tctccaaagc caaagggcag 1020
ccccgagaac cacagggtgtg caccctgccc ccatcccggg atgagctgac caagaaccag 1080
gtcagcctct cgtgcgcagt caaaggcttc tatcccagcg acatgcgcgt ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacgcctc cgtgctgga ctccgacggc 1200
tccttcttcc tcgtgagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtaaa 1335

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<210> SEQ ID NO 221
<211> LENGTH: 591
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47 GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

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<400> SEQUENCE: 221

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100         105         110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115         120         125
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130         135         140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145         150         155         160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165         170         175
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180         185         190
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195         200         205
Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210         215         220
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu
225         230         235         240

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Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
				245					250					255		
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
			260					265					270			
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
		275					280					285				
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
	290					295					300					
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
305				310						315					320	
Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
			325					330						335		
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Cys	
			340					345					350			
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu	Val	Lys	
		355					360					365				
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	
	370					375					380					
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	
385				390						395					400	
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	
			405					410						415		
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	
			420					425					430			
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	
		435					440					445				
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Pro	Ala	Ser	Ser	Ser	
	450					455					460					
Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	Leu	Gln	
465				470						475					480	
Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	Thr	Arg	
			485					490						495		
Met	Leu	Thr	Ala	Lys	Phe	Ala	Met	Pro	Lys	Lys	Ala	Thr	Glu	Leu	Lys	
			500					505					510			
His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val	Leu	
		515						520				525				
Asn	Gly	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	Leu	Ile	
	530					535					540					
Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	Thr	Thr	
545				550						555					560	
Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr	Ile	Val	Glu	Phe	Leu	
				565				570						575		
Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser	Ile	Ile	Ser	Thr	Leu	Thr		
			580					585					590			

&lt;210&gt; SEQ ID NO 222

&lt;211&gt; LENGTH: 1773

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: DP47 GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 222

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct 120

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ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtgtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc 300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgtctag caccaagggc 360
ccatcggtct tccccctggc accctcctcc aagagcacct ctgggggcac agcggccctg 420
ggctgccttg tcaaggacta ctccccgaa ccggtgacgg tgctgtggaa ctcaggcgcc 480
ctgaccagcg gcgtgcacac ctccccgct gtcctacagt cctcaggact ctactccctc 540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg 600
aatcacaagc ccagcaacac caaggtggac aagaaagtgt agcccaaatc ttgtgacaaa 660
actcacacat gcccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttctc 720
ttcccccaa aaccaagga caccctcatg atctcccgga cccctgaggt cacatgcgtg 780
gtggtggacg tgagccacga agaccctgag gtcaagtcca actgggtacgt ggacggcgtg 840
gaggtgcata atgccaagac aaagccgagg gaggagcagt acaacagcac gtaccgtgtg 900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcag 960
gtctccaaca aagccctcgg cgcctccatc gagaaaacca tctccaaagc caaagggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag 1080
gtcagcctgt ggtgcctggt caaaggcttc tatccagcg acatgccgtt ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc 1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtggcgccgg aggctccgga ggcggaggtt ctggcggagg tggcgctcct 1380
gcctcctcca gcaccaagaa aaccagctc cagctggaac atctcctgct ggatctgcag 1440
atgatcctga acggcatcaa caactacaag aacccaagc tgaccggat gctgaccgcc 1500
aagttcgcca tgccaagaa ggcaccgag ctgaaacatc tgcagtgcct ggaagaggaa 1560
ctgaagcctc tggaagaggt gctgaacggc gccagtccta agaacttcca cctgaggcct 1620
cgggacctga tctcaacat caacgtgatc gtgctggaac tgaagggtc cgagacaacc 1680
ttcatgtgcy agtacgccga cgagacagct accatcgtgg aatttctgaa ccggtggatc 1740
accttcgccc agtccatcat ctccaccctg acc 1773

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<210> SEQ ID NO 223
<211> LENGTH: 591
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GL Fab HC-Fc knob (LALA P329G)- IL-2 wt

<400> SEQUENCE: 223

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
          50          55          60

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Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr	65	70	75	80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	85	90	95	
Ala	Lys	Gly	Ser	Gly	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	100	105	110	
Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	115	120	125	
Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	130	135	140	
Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	145	150	155	160
Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	165	170	175	
Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	180	185	190	
Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	195	200	205	
Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	210	215	220	
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val	Phe	Leu	225	230	235	240
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	245	250	255	
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	260	265	270	
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	275	280	285	
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	290	295	300	
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	305	310	315	320
Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	325	330	335	
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Cys	340	345	350	
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu	Val	Lys	355	360	365	
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	370	375	380	
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	385	390	395	400
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	405	410	415	
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	420	425	430	
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	435	440	445	
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Pro	Thr	Ser	Ser	Ser	450	455	460	
Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	Leu	Gln	465	470	475	480
Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	Thr	Arg				

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485							490					495				
Met	Leu	Thr	Phe	Lys	Phe	Tyr	Met	Pro	Lys	Lys	Ala	Thr	Glu	Leu	Lys	
			500					505					510			
His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val	Leu	
		515					520					525				
Asn	Leu	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	Leu	Ile	
	530					535					540					
Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	Thr	Thr	
545					550					555					560	
Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr	Ile	Val	Glu	Phe	Leu	
				565					570					575		
Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser	Ile	Ile	Ser	Thr	Leu	Thr		
			580					585					590			

&lt;210&gt; SEQ ID NO 224

&lt;211&gt; LENGTH: 1773

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: DP47GS Fab HC-Fc knob (LALA P329G) - IL-2 wt

&lt;400&gt; SEQUENCE: 224

```

gagggtgcaat tgttgagagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccagggt      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagccctgag agccgaggac acggccgtat attactgtgc gaaaggcagc      300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgtctag caccaagggc      360
ccatcggtct tccccctggc accctcctcc aagagcacct ctgggggcac agcggccctg      420
ggctgcctgg tcaaggacta ctccccgaa ccggtgacgg tgctgtggaa ctcaggcgcc      480
ctgaccagcg gcgtgcacac ctccccggt gtcctacagt cctcaggact ctactccctc      540
agcagcgctg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg      600
aatcacaagc ccagcaacac caaggtggac aagaaagtgt agcccaaata ttgtgacaaa      660
actcacacat gcccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttctct      720
ttcccccaa aaccacaagga caccctcatg atctcccga cccctgaggt cacatgcgtg      780
gtggtggacg tgagccacga agaccctgag gtcaagtcca actgggtacgt ggacggcgtg      840
gagggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg      900
gtcagcgctc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcag      960
gtctccaaca aagccctcgg cgcccccatc gagaaaacca tctccaaagc caaagggcag      1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag      1080
gtcagcctgt ggtgcctggt caaaggcttc tatcccagcg acatcgccgt ggagtgggag      1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc      1200
tccttcttcc tctacagcaa gctcacgtg gacaagagca ggtggcagca ggggaacgtc      1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc      1320
ctgtctccgg gtggcgcggt aggtccgga ggcggaggtt ctggcgaggg tggcgctcct      1380
acatcctcca gcaccaagaa aaccagctc cagctggaac atctcctgct ggatctgcag      1440
atgatcctga acggcatcaa caactacaag aacccaagc tgaccggat gctgaccttc      1500

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aagttctaca tgcccaagaa ggccaccgag ctgaaacatc tgcagtgcct ggaagaggaa 1560
ctgaagcctc tggaagaggt gctgaacctg gcccagtgcca agaacttcca cctgaggcct 1620
cgggacctga tctccaacat caacgtgata gtgctggaac tgaagggctc cgagacaacc 1680
ttcatgtgcg agtacgcga cgagacagct accatcgtgg aatttctgaa cgggtggatc 1740
accttcgccc agtccatcat ctccaccctg acc 1773

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<210> SEQ ID NO 225
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS Fab LC

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<400> SEQUENCE: 225

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Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1             5             10            15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20            25            30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35            40            45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50            55            60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65            70            75            80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85            90            95
Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100           105           110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115           120           125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130           135           140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145           150           155           160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165           170           175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180           185           190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195           200           205
Ser Phe Asn Arg Gly Glu Cys
210           215

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<210> SEQ ID NO 226
<211> LENGTH: 645
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS Fab LC

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<400> SEQUENCE: 226

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gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtc gagtgtagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180

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gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagtatggta gctcaccgct gacgttcggc 300
caggggacca aagtggaaat caaacgtacg gtggctgcac catctgtctt catcttcccg 360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgectgct gaataacttc 420
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcacccctg 540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag 600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 645

```

&lt;210&gt; SEQ ID NO 227

&lt;211&gt; LENGTH: 451

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: CH1A1A Fab HC-Fc hole (wt)

&lt;400&gt; SEQUENCE: 227

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20     25     30
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35     40     45
Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50     55     60
Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65     70     75     80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85     90     95
Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100    105    110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115    120    125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130    135    140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145    150    155    160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165    170    175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180    185    190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195    200    205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210    215    220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225    230    235    240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245    250    255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260    265    270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275    280    285

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His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300  
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 325 330 335  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350  
 Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser  
 355 360 365  
 Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 370 375 380  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 385 390 395 400  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val  
 405 410 415  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 420 425 430  
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 435 440 445  
 Pro Gly Lys  
 450

&lt;210&gt; SEQ ID NO 228

&lt;211&gt; LENGTH: 1353

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: CH1A1A Fab HC-Fc hole (wt)

&lt;400&gt; SEQUENCE: 228

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg	60
tcctgcgaagg ccagcgggcta caccttcacc gagttcggca tgaactgggt cgcacaggct	120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac	180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac	240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac	300
ttcgctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct	360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc	420
ggcggaacag ccgccctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg	480
tcctggaaca gcgagccct gaccagcggc gtgcacacct ttccagccgt gctgcagagc	540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcaccag	600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag	660
cccaagagct ggcacaagac ccacacctgt ccccttgctc ctgcccctga gctgctgggc	720
ggaccacagc tgttcctgtt cccccaaag cccaaggaca ccctgatgat cagccggacc	780
cccgaagtga cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagttcaat	840
tggtagctgg acggcgtgga ggtgcacaat gccaaagacca agccccggga ggaacagtac	900
aacagcacct accgggtggt gtcctgctg accgtgctgc accaggactg gctgaacggc	960
aaagagtaca agtgcaaggt ctccaacaag gccctgcctg ccccatcga gaaaaccatc	1020
agcaaggcca agggccagcc cagagaaccc caggtgtgca ccctgcccc cagcagagat	1080

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gagctgacca agaaccaggt gtccttgagc tgtgccgtca agggcttcta ccccgagcat 1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct 1200
gtgctggaca gcgacggcag cttcttcttg gtgtccaaac tgacctgga caagagccgg 1260
tggcagcagg gcaacgtggt cagctgcagc gtgatgcacg aggccttgca caaccactac 1320
accagaagt ccctgagcct gagccccggc aag 1353

```

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<210> SEQ ID NO 229
<211> LENGTH: 599
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CH1A1A Fab HC-Fc knob (wt) -IL-2 qm

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<400> SEQUENCE: 229

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20          25          30
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35          40          45
Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50          55          60
Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65          70          75          80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100         105         110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115         120         125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130         135         140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145         150         155         160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165         170         175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180         185         190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195         200         205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210         215         220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225         230         235         240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245         250         255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260         265         270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275         280         285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290         295         300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly

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305	310	315	320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile	325	330	335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val	340	345	350
Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser	355	360	365
Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu	370	375	380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro	385	390	400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val	405	410	415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met	420	425	430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser	435	440	445
Pro Gly Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly	450	455	460
Gly Gly Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu	465	470	480
Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn	485	490	495
Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met	500	505	510
Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu	515	520	525
Leu Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe	530	535	540
His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu	545	550	555
Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu	565	570	575
Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln	580	585	590
Ser Ile Ile Ser Thr Leu Thr	595		

&lt;210&gt; SEQ ID NO 230

&lt;211&gt; LENGTH: 1797

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: CH1A1A Fab HC-Fc knob (wt) -IL-2 qm

&lt;400&gt; SEQUENCE: 230

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg	60
tcctgcaagg ccagcggtcta caccttcacc gagttcggca tgaactgggt ccgacaggct	120
ccaggccagg gcctcgaaatg gatgggctgg atcaacacca agaccggcga ggccacctac	180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac	240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac	300
ttgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct	360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc	420

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ggcggaacag cgcacctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg 480
tcctggaaca gcgagagccct gaccagcggc gtgcacacct ttccagccgt gctgcagagc 540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcaccacg 600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag 660
cccaagagct gcgacaagac ccacacctgt ccccttggtc ctgcccctga gctgctgggc 720
ggaccacagc tgttctgtgt ccccccaaag cccaaggaca ccctgatgat cagccggacc 780
cccgaaagtg cctgcgtggt ggtggacgtg tcccacgagg acctgaagt gaagtccaat 840
tggtacgtgg acggcgtgga ggtgcacaat gccaaagacca agccccggga ggaacagtac 900
aacagcacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc 960
aaagagtaca agtgcaaggt ctccaacaag gccctgcctg ccccatcgaa gaaaaccatc 1020
agcaaggcca agggccagcc cagagaaccc caggtgtaca ccctgcccc ctgcagagat 1080
gagctgacca agaaccaggt gtccctgtgg tgtctggta agggcttcta cccagcgat 1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct 1200
gtgctggaca gcgacggcag cttcttctg tactccaaac tgaccgtgga caagagccgg 1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggcctgca caaccactac 1320
accagaagt ccctgagcct gagccccggc aagtccggag gcggaggctc cggcggggga 1380
ggttctggcg gaggtggcgc tcctgcctcc tccagcacca agaaaaccca gctccagctg 1440
gaacatctcc tgctggatct gcagatgate ctgaacggca tcaacaacta caagaacccc 1500
aagctgaccc ggatgctgac cgccaagttc gccatgcccc agaaggccac cgagctgaaa 1560
catctgcagt gcctggaaga ggaactgaag cctctggaag aggtgctgaa cggcgcccag 1620
tccaagaact tccacctgag gcctcgggac ctgatctcca acatcaacgt gatcgtgctg 1680
gaactgaagg gctccgagac aaccttcacg tgcgagtacg ccgacgagac agctaccatc 1740
gtggaatttc tgaaccggtg gatcaccttc gccagtcaca tcatctccac cctgacc 1797

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&lt;210&gt; SEQ ID NO 231

&lt;211&gt; LENGTH: 215

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2F1 Fab LC

&lt;400&gt; SEQUENCE: 231

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1             5             10             15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
20            25            30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35            40            45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
50            55            60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65            70            75            80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
85            90            95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
100           105           110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser

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115	120	125
Gly Thr Ala Ser Val Val	Cys Leu Leu Asn Asn	Phe Tyr Pro Arg Glu
130	135	140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser		
145	150	155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu		
	165 170	175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val		
	180 185	190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys		
	195 200	205
Ser Phe Asn Arg Gly Glu Cys		
210	215	

<210> SEQ ID NO 232  
 <211> LENGTH: 645  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2F1 Fab LC

<400> SEQUENCE: 232

```

gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc      60
atcacttgca aggccagtg cgcgtgtgggt acgtatgttg cgtgggtatca gcagaaacca      120
gggaaagcac ctaagctcct gatctattcg gcatcctacc gcaaaagggg agtcccatca      180
aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct      240
gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc      300
cagggcacca agctcgagat caagcgtacg gtggctgcac catctgtctt catcttcccg      360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgctgct gaataacttc      420
tatccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc      480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcacccctg      540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag      600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgtt      645

```

<210> SEQ ID NO 233  
 <211> LENGTH: 451  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10 Fab HC-Fc knob (LALA P329G)

<400> SEQUENCE: 233

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser	
1 5 10 15	
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr	
20 25 30	
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met	
35 40 45	
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe	
50 55 60	
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr	
65 70 75 80	
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	

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Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125  
 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140  
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160  
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175  
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190  
 Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
 195 200 205  
 Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220  
 Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly  
 225 230 235 240  
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 245 250 255  
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 260 265 270  
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 275 280 285  
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300  
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile  
 325 330 335  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350  
 Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser  
 355 360 365  
 Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 370 375 380  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 385 390 395 400  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 405 410 415  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 420 425 430  
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 435 440 445  
 Pro Gly Lys  
 450

&lt;210&gt; SEQ ID NO 234

&lt;211&gt; LENGTH: 1353

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc knob (LALA P329G)

&lt;400&gt; SEQUENCE: 234

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cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcttgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tcagctagca ccaagggccc atcggctctc cccctggcac cctcctccaa gagcacctct      420
gggggcacag cggccctggg ctgcctggtc aaggactact tcccgaacc ggtgacggtg      480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggtgt cctacagtcc      540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcaccacg      600
acctacatct gcaacgtgaa tcacaagccc agcaaacacca aggtggacaa gaaagttgag      660
cccaaattct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttctctct cccccaaaa cccaaggaca ccctcatgat ctcccgacc      780
cctgaggtca catgctggtt ggtggacgtg agccacgaag accctgaggt caagttcaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaca agccgcggga ggagcagtac      900
aacagcacgt accgtgtggt cagcgtctc accgtctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcgga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atgccgggat     1080
gagctgacca agaaccaggt cagcctgtgg tgcctggtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttctctctc tacagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggtctctga caaccactac     1320
acgcagaaga gcctctccct gtctccgggt aaa                                     1353

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&lt;210&gt; SEQ ID NO 235

&lt;211&gt; LENGTH: 805

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)-scIL-10

&lt;400&gt; SEQUENCE: 235

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1           5           10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20          25          30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35          40          45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50          55          60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100         105         110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser

```

	115					120					125				
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
	130					135					140				
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
145					150					155					160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala
				165					170					175	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
			180					185					190		
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His
			195				200					205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys
	210					215					220				
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly
225					230					235					240
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
				245					250					255	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
			260					265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
305					310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile
				325					330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
			340					345					350		
Cys	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
		355					360					365			
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
385					390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val
				405					410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
		435					440					445			
Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	450					455					460				
Ser	Ser	Pro	Gly	Gln	Gly	Thr	Gln	Ser	Glu	Asn	Ser	Cys	Thr	His	Phe
465					470					475					480

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Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly  
 545 550 555 560

Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe  
 565 570 575

Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala  
 580 585 590

Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe  
 595 600 605

Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg  
 610 615 620

Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 625 630 635 640

Gly Gly Gly Gly Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser  
 645 650 655

Cys Thr His Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg  
 660 665 670

Asp Ala Phe Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu  
 675 680 685

Asp Asn Leu Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr  
 690 695 700

Leu Gly Cys Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu  
 705 710 715 720

Val Met Pro Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val  
 725 730 735

Asn Ser Leu Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg  
 740 745 750

Cys His Arg Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln  
 755 760 765

Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala  
 770 775 780

Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr  
 785 790 795 800

Met Lys Ile Arg Asn  
 805

&lt;210&gt; SEQ ID NO 236

&lt;211&gt; LENGTH: 2415

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)-scIL-10

&lt;400&gt; SEQUENCE: 236

cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60

tcctgcgaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120

cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac 180

gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac accgcctgtg attactgtgc gagactgtac 300

ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360

tcagctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct 420

gggggcacag cgccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccgctgtg cctacagtcc 540

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tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcaccacag	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg	720
ggaccgtcag tcttctctct cccccaaaa cccaaggaca ccctcatgat ctcccgacc	780
cctgaggtea catgctgtgt ggtggacgtg agccacgaag accctgaggt caagttcaac	840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaca agccgcggga ggagcagtac	900
aacagcacgt accgtgttgt cagcgtcttc accgtctgc accaggactg gctgaatggc	960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc	1020
tccaagcca aagggcagcc ccgagaacca caggtgtgca ccctgcccc atcccggtat	1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggtctcta tcccagcgac	1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc	1200
gtgctggact ccgacggctc cttcttcttc gtgagcaagc tcaccgtgga caagagcagg	1260
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggtcttgca caaccactac	1320
acgcagaaga gcctctccct gtctccgggt ggccggcgag gctccggagg cggaggatct	1380
gggggaggcg gaagtagccc gggccagggc acccagagcg agaacagctg caccacttc	1440
cccggaacc tgcccaacat gctgcgggac ctgagggacg ccttcagcag agtgaaaacc	1500
ttcttcaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat	1560
ttcaagggt acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa	1620
gtgatgcccc agggcgagaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc	1680
gagaacctga aaacctgcg gctgagactg cggcgggtgcc acagatttct gccctgcgag	1740
aacaagagca aggccgtgga acaggtgaag aacgccttca acaagctgca ggaaaagggc	1800
atctacaagg ccattgtcca gttcgacatc ttcatcaact acatcgaagc ttacatgacc	1860
atgaagatca gaaacggcgg aggcggatct ggccggcgtg gaagtggagg cggaggatct	1920
gggggaggcg gaagtagccc gggccagggc acccagagcg agaacagctg caccacttc	1980
cccggaacc tgcccaacat gctgcgggac ctgagggacg ccttcagcag agtgaaaacc	2040
ttcttcaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat	2100
ttcaagggt acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa	2160
gtgatgcccc agggcgagaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc	2220
gagaacctga aaacctgcg gctgagactg cggcgggtgcc acagatttct gccctgcgag	2280
aacaagagca aggccgtgga acaggtgaag aacgccttca acaagctgca ggaaaagggc	2340
atctacaagg ccattgtcca gttcgacatc ttcatcaact acatcgaggc ctacatgaca	2400
atgaaaatcc gcaat	2415

&lt;210&gt; SEQ ID NO 237

&lt;211&gt; LENGTH: 631

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)-IL-10M1

&lt;400&gt; SEQUENCE: 237

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1                    5                    10                    15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr

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20							25					30				
Ala	Ile	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	
		35					40					45				
Gly	Gly	Ile	Ile	Pro	Ile	Phe	Gly	Thr	Ala	Asn	Tyr	Ala	Gln	Lys	Phe	
	50					55					60					
Gln	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr	
	65				70				75						80	
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
			85						90					95		
Ala	Arg	Leu	Tyr	Gly	Tyr	Ala	Tyr	Tyr	Gly	Ala	Phe	Asp	Tyr	Trp	Gly	
			100					105					110			
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	
		115					120					125				
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	
	130					135					140					
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	
	145				150					155					160	
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	
			165						170					175		
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	
		180						185					190			
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	
		195					200					205				
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	
	210				215						220					
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly	
	225				230					235					240	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	
			245						250					255		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	
		260						265					270			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	
		275					280					285				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	
	290				295						300					
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	
	305				310					315					320	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	
			325						330					335		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	
		340						345					350			
Cys	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	
		355					360					365				
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	
		370				375					380					
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	
	385				390					395					400	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	
			405						410					415		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	
			420					425					430			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	
		435					440						445			

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Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 450 455 460  
 Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe  
 465 470 475 480  
 Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser  
 485 490 495  
 Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu  
 500 505 510  
 Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln  
 515 520 525  
 Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln  
 530 535 540  
 Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly  
 545 550 555 560  
 Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe  
 565 570 575  
 Leu Pro Cys Glu Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val  
 580 585 590  
 Glu Gln Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr  
 595 600 605  
 Lys Ala Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr  
 610 615 620  
 Met Thr Met Lys Ile Arg Asn  
 625 630

<210> SEQ ID NO 238  
 <211> LENGTH: 1893  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)-IL-10M1  
 <400> SEQUENCE: 238

cagggtcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60  
 tcttgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120  
 cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaaactac 180  
 gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240  
 atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300  
 ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360  
 tcagctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct 420  
 gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480  
 tcgtggaact caggccctc gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540  
 tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag 600  
 acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660  
 cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg 720  
 ggaccgtcag tcttctctct ccccccaaaa cccaaggaca ccctcatgat ctcccgacc 780  
 cctgaggtca catgctggtt ggtggacgtg agccacgaag accctgaggt caagttcaac 840  
 tggtagctgg acggcgtgga ggtgcataat gccaaagaca agccgcggga ggagcagtac 900  
 aacagcacgt accgtgtggt cagcgtcttc accgtctgc accaggactg gctgaatggc 960

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aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcgaa gaaaaccatc 1020
tccaaagcca aaggcgagcc ccgagaacca caggtgtgca ccctgcccc atcccgggat 1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac 1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc 1200
gtgctggact ccgacggctc cttcttctc gtgagcaagc tcaccgtgga caagagcagg 1260
tggcagcagg ggaacgtott ctcatgctcc gtgatgcatg aggctctgca caaccactac 1320
acgcagaaga gcctctccct gtctccgggt ggcggcggag gctccggagg cggaggaagt 1380
ggcggcgggt gcagctctcc aggccagggc acccagagcg agaacagctg caccacttc 1440
cccgcaacc tgcccaacat gctgctggac ctgagggacg ccttcagcag agtgaaaacc 1500
ttcttcaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat 1560
ttcaagggt acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa 1620
gtgatgcccc aggcgagaaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc 1680
gagaacctga aaacctgctg gctgagactg cggcgggtgcc acagatttct gccctgcgag 1740
aacggcggag gctctggcgg aaagtccaag gccgtggaac aggtgaagaa cgccttcaac 1800
aagctgcagg aaaagggcat ctacaaggcc atgagcgagt tcgacatctt catcaactac 1860
atcgaagctt acatgacaat gaagatacga aac 1893

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&lt;210&gt; SEQ ID NO 239

&lt;211&gt; LENGTH: 214

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab LC

&lt;400&gt; SEQUENCE: 239

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
          20           25           30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
          35           40           45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
          65           70           75           80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
          85           90           95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
          100          105          110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
          115          120          125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
          130          135          140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
          145          150          155          160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
          165          170          175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
          180          185          190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser

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195	200	205	
Phe Asn Arg Gly Glu Cys			
210			
 <210> SEQ ID NO 240			
<211> LENGTH: 642			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: 2B10 Fab LC			
 <400> SEQUENCE: 240			
gatatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc			60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca			120
gggaaagccc ctaagcgct gatctatgct gcattcagtt tgcagagtgg cgtcccatca			180
aggttcagcg gcggtggatc cgggacagag ttactctca ccatcagcag cttgcagcct			240
gaagattttg ccacctatta ctgcttgagc aatggtctgc agcccgcgac gtttgccag			300
ggcaccaaaag tcgagatcaa gcgtacggtg gctgcacat ctgtcttcat cttcccgcca			360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat			420
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag			480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg			540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc			600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt			642
 <210> SEQ ID NO 241			
<211> LENGTH: 447			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: 4G8 Fab HC-Fc knob (LALA P329G)			
 <400> SEQUENCE: 241			
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly			
1 5 10 15			
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr			
20 25 30			
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
35 40 45			
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val			
50 55 60			
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
65 70 75 80			
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
85 90 95			
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu			
100 105 110			
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu			
115 120 125			
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys			
130 135 140			
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser			
145 150 155 160			
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser			
165 170 175			

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Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser  
 180 185 190  
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn  
 195 200 205  
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His  
 210 215 220  
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val  
 225 230 235 240  
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr  
 245 250 255  
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu  
 260 265 270  
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys  
 275 280 285  
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser  
 290 295 300  
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys  
 305 310 315 320  
 Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile  
 325 330 335  
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro  
 340 345 350  
 Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu  
 355 360 365  
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
 370 375 380  
 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser  
 385 390 395 400  
 Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
 405 410 415  
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu  
 420 425 430  
 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 435 440 445

&lt;210&gt; SEQ ID NO 242

&lt;211&gt; LENGTH: 1341

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4G8 Fab HC-Fc knob (LALA P329G)

&lt;400&gt; SEQUENCE: 242

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gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgccg tgagctgggt ccgccaggct      120
ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactcgc tgaaggccgc gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc      360
aagggcccat cggtcttccc cctggcacc cctccaaga gcacctctgg gggcacagcg      420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca      480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac      540

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tcctcagca gcgtggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc   600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt   660
gacaaaaactc acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc   720
ttcctcttcc ccccaaaacc caaggacacc ctcctgatct cccggacccc tgaggtcaca   780
tgcggtggtgg tggacgtgag ccacgaagac cctgagggtca agttcaactg gtacgtggac   840
ggcggtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac   900
cgtgtgtgtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag   960
tgcaagggtct ccaacaaagc cctcggcgcc cccatcgaga aaacctctc caaagccaaa  1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag  1080
aaccagggtca gcctgtggtg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag  1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccggt gctggactcc  1200
gacggctcct tcttcctcta cagcaagctc accgtggaca agagcagggtg gcagcagggg  1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc  1320
ctctccctgt ctccgggtaa a                                     1341

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<210> SEQ ID NO 243
<211> LENGTH: 801
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)-scIL-10

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<400> SEQUENCE: 243

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100         105         110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115         120         125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130         135         140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145         150         155         160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165         170         175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180         185         190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195         200         205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His

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210	215	220
Thr Cys Pro Pro Cys Pro	Ala Pro Glu Ala Ala	Gly Gly Pro Ser Val
225	230	235 240
Phe Leu Phe Pro Pro Lys Pro	Lys Asp Thr Leu Met Ile Ser Arg Thr	
	245	250 255
Pro Glu Val Thr Cys Val Val	Val Asp Val Ser His Glu Asp Pro Glu	
	260	265 270
Val Lys Phe Asn Trp Tyr Val	Asp Gly Val Glu Val His Asn Ala Lys	
	275	280 285
Thr Lys Pro Arg Glu Glu Gln	Tyr Asn Ser Thr Tyr Arg Val Val Ser	
	290	295 300
Val Leu Thr Val Leu His Gln	Asp Trp Leu Asn Gly Lys Glu Tyr Lys	
	305	310 315 320
Cys Lys Val Ser Asn Lys Ala	Leu Gly Ala Pro Ile Glu Lys Thr Ile	
	325	330 335
Ser Lys Ala Lys Gly Gln Pro	Arg Glu Pro Gln Val Cys Thr Leu Pro	
	340	345 350
Pro Ser Arg Asp Glu Leu Thr	Lys Asn Gln Val Ser Leu Ser Cys Ala	
	355	360 365
Val Lys Gly Phe Tyr Pro Ser	Asp Ile Ala Val Glu Trp Glu Ser Asn	
	370	375 380
Gly Gln Pro Glu Asn Asn Tyr	Lys Thr Thr Pro Pro Val Leu Asp Ser	
	385	390 395 400
Asp Gly Ser Phe Phe Leu Val	Ser Lys Leu Thr Val Asp Lys Ser Arg	
	405	410 415
Trp Gln Gln Gly Asn Val Phe	Ser Cys Ser Val Met His Glu Ala Leu	
	420	425 430
His Asn His Tyr Thr Gln Lys	Ser Leu Ser Leu Ser Pro Gly Gly Gly	
	435	440 445
Gly Gly Ser Gly Gly Gly Gly	Ser Gly Gly Gly Gly Ser Ser Pro Gly	
	450	455 460
Gln Gly Thr Gln Ser Glu Asn	Ser Cys Thr His Phe Pro Gly Asn Leu	
	465	470 475 480
Pro Asn Met Leu Arg Asp Leu	Arg Asp Ala Phe Ser Arg Val Lys Thr	
	485	490 495
Phe Phe Gln Met Lys Asp Gln	Leu Asp Asn Leu Leu Lys Glu Ser	
	500	505 510
Leu Leu Glu Asp Phe Lys Gly	Tyr Leu Gly Cys Gln Ala Leu Ser Glu	
	515	520 525
Met Ile Gln Phe Tyr Leu Glu	Glu Val Met Pro Gln Ala Glu Asn Gln	
	530	535 540
Asp Pro Asp Ile Lys Ala His	Val Asn Ser Leu Gly Glu Asn Leu Lys	
	545	550 555 560
Thr Leu Arg Leu Arg Leu Arg	Arg Cys His Arg Phe Leu Pro Cys Glu	
	565	570 575
Asn Lys Ser Lys Ala Val Glu	Gln Val Lys Asn Ala Phe Asn Lys Leu	
	580	585 590
Gln Glu Lys Gly Ile Tyr Lys	Ala Met Ser Glu Phe Asp Ile Phe Ile	
	595	600 605
Asn Tyr Ile Glu Ala Tyr Met	Thr Met Lys Ile Arg Asn Gly Gly Gly	
	610	615 620
Gly Ser Gly Gly Gly Gly Ser	Gly Gly Gly Gly Ser Gly Gly Gly Gly	
	625	630 635 640

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Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe  
645 650 655

Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser  
660 665 670

Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu  
675 680 685

Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln  
690 695 700

Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln  
705 710 715 720

Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly  
725 730 735

Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe  
740 745 750

Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala  
755 760 765

Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe  
770 775 780

Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg  
785 790 795 800

Asn

<210> SEQ ID NO 244  
<211> LENGTH: 2403  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)-scIL-10

<400> SEQUENCE: 244

gaggtgcaat tgttgtagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc	60
tcctgtgcag cctccgatt cacctttagc agttatgccca tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggtag	300
ctgggtaatt ttgactactg gggccaagga accctgggtc cgtctcagag tgctagcacc	360
aagggcccat cgggtcttccc cctggcacc cctctccaaga gcacctctgg gggcacagcg	420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca	480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac	540
tccctcagca gcgtgggtgac cgtgcctccc agcagcttgg gcaccagac ctacatctgc	600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt	660
gacaaaaactc acacatgccc accgtgccc gacacctgaag ctgcaggggg accgtcagtc	720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca	780
tgcggtggtag tggacgtgag ccacgaagac cctgagggtc agttcaactg gtacgtggac	840
ggcgtaggag tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac	900
cgtgtgtgtc gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag	960
tgcaaggtct ccaacaaagc cctcgggccc cccatcgaga aaaccatctc caaagccaaa	1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag	1080

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aaccaggtea gcctctcgtg cgcagtc aaa ggcttctatc ccagcgacat cgcctggag 1140
tgaggagaca atgggcagcc ggagaacaac tacaagacca cgcctcccggt gctggactcc 1200
gacggctcct tcttctcgtg gagcaagctc accgtggaca agagcagggtg gcagcagggg 1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggatctgg gggaggcgga 1380
agtagcccg ggcagggcac ccagagcgag aacagctgca cccacttccc cggcaacctg 1440
cccaacatgc tgcgggacct gagggacgcc ttcagcagag tgaaaacctt cttccagatg 1500
aaggaccagc tggacaacct gctgctgaaa gagagcctgc tggaagattt caagggtac 1560
ctgggctgtc aggccctgag cgagatgac cagttctacc tggaagaagt gatgcccag 1620
gccgagaacc aggaccccg catcaaggcc cactgaaca gcctgggcga gaacctgaaa 1680
acctgcggc tgagactgag cgggtgccac agatttctgc cctgcgagaa caagagcaag 1740
gccgtggaac aggtgaagaa cgccttcaac aagctgcagg aaaagggcac ctacaaggcc 1800
atgtccgagt tgcacatctt catcaactac atcgaagctt acatgacat gaagatcaga 1860
aacggcggag cggatctg cggcggtgga agtgaggcg gaggatctgg gggaggcgga 1920
agtagcccg ggcagggcac ccagagcgag aacagctgca cccacttccc cggcaacctg 1980
cccaacatgc tgcgggacct gagggacgcc ttcagcagag tgaaaacctt cttccagatg 2040
aaggaccagc tggacaacct gctgctgaaa gagagcctgc tggaagattt caagggtac 2100
ctgggctgtc aggccctgag cgagatgac cagttctacc tggaagaagt gatgcccag 2160
gccgagaacc aggaccccg catcaaggcc cactgaaca gcctgggcga gaacctgaaa 2220
acctgcggc tgagactgag cgggtgccac agatttctgc cctgcgagaa caagagcaag 2280
gccgtggaac aggtgaagaa cgccttcaac aagctgcagg aaaagggcac ctacaaggcc 2340
atgtccgagt tgcacatctt catcaactac atcgaggcct acatgacaat gaaaatccgc 2400
aat 2403

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<210> SEQ ID NO 245
<211> LENGTH: 627
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)-IL-10M1

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<400> SEQUENCE: 245

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```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20        25        30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35        40        45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50        55        60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65        70        75        80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85        90        95
Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100       105       110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115       120       125

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Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
130						135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155					160
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
				165					170						175
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser
			180					185					190		
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
		195					200					205			
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His
	210					215					220				
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val
225					230					235					240
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
				245					250						255
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu
			260					265					270		
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
		275					280					285			
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
		290				295					300				
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
305					310					315					320
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile
				325					330						335
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu	Pro
			340					345					350		
Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Ala
		355					360					365			
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380				
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385					390					395					400
Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
				405					410						415
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
			420					425					430		
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly
		435					440					445			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Pro	Gly
	450					455					460				
Gln	Gly	Thr	Gln	Ser	Glu	Asn	Ser	Cys	Thr	His	Phe	Pro	Gly	Asn	Leu
465					470					475					480
Pro	Asn	Met	Leu	Arg	Asp	Leu	Arg	Asp	Ala	Phe	Ser	Arg	Val	Lys	Thr
				485					490						495
Phe	Phe	Gln	Met	Lys	Asp	Gln	Leu	Asp	Asn	Leu	Leu	Leu	Lys	Glu	Ser
			500					505					510		
Leu	Leu	Glu	Asp	Phe	Lys	Gly	Tyr	Leu	Gly	Cys	Gln	Ala	Leu	Ser	Glu
		515					520					525			
Met	Ile	Gln	Phe	Tyr	Leu	Glu	Glu	Val	Met	Pro	Gln	Ala	Glu	Asn	Gln
	530					535					540				

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Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu Asn Leu Lys  
545 550 555 560

Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys Glu  
565 570 575

Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val Glu Gln Val Lys  
580 585 590

Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser  
595 600 605

Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys  
610 615 620

Ile Arg Asn  
625

<210> SEQ ID NO 246

<211> LENGTH: 1881

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: 4G8 Fab HC-Fc hole (LALA P329G)-IL-10M1

<400> SEQUENCE: 246

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc   60
tcctgtgcag cctccgatt cacctttagc agttatgccca tgagctgggt ccgccaggct   120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac   180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat   240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggtag   300
ctgggtaatt ttgactactg gggccaagga accctggtea ccgtctcgag tgctagcacc   360
aagggcccat cggtcttccc cctggcaccc tctccaaga gcacctctgg gggcacagcg   420
gccctgggct gcctggtaaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca   480
ggcgccctga ccagcggcgt gcacaccttc ccggtgtgac tacagtcttc aggactctac   540
tccctcagca gcgtgggtgac cgtgcctccc agcagcttgg gcaccagac ctacatctgc   600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt   660
gacaaaaact acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc   720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca   780
tgcggtgggg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac   840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac   900
cgtgtggtea gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag   960
tgcaaggtct ccaacaaagc cctcgcgccc cccatcgaga aaaccatctc caaagccaaa  1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag  1080
aaccaggtea gcctctctgt cgcagtcaaa ggcttctatc ccagcgacat cgccgtggag  1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccggt gctggactcc  1200
gacggctcct tcttctcgtg gagcaagctc accgtggaca agagcagggt gcagcagggg  1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc  1320
ctctccctgt ctccgggtgg cggcgagggc tccggaggcg gaggaagtgg cggcggtggc  1380
agctctccag gccagggcac ccagagcgag aacagctgca cccacttccc cggcaacctg  1440
cccaacatgc tgcgggacct gagggacgcc ttcagcagag tgaaaacctt cttccagatg  1500
aaggaccagc tggacaacct gctgctgaaa gagagcctgc tggaagattt caagggtac  1560

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ctgggctgtc aggccctgag cgagatgac cagttctacc tggaagaagt gatgccccag 1620
gccgagaacc aggaccccca catcaaggcc cacgtgaaca gcctgggcga gaacctgaaa 1680
accctgcggc tgagactgcg gcggtgccac agatttctgc cctgcgagaa cggcggaggc 1740
tctggcggaa agtccaaggc cgtggaacag gtgaagaacg ccttcaacaa gctgcaggaa 1800
aagggcatct acaaggccat gagcaggttc gacatcttca tcaactacat cgaagcttac 1860
atgacaatga agatacgaaa c 1881

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&lt;210&gt; SEQ ID NO 247

&lt;211&gt; LENGTH: 366

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IL-2 qm-Fc knob (LALA P329G) (IL-2 N-terminal)

&lt;400&gt; SEQUENCE: 247

```

Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1      5      10      15
Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20     25     30
Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys
35     40     45
Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
50     55     60
Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu
65     70     75     80
Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85     90     95
Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100    105    110
Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile
115    120    125
Ile Ser Thr Leu Thr Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr
130    135    140
Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
145    150    155    160
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
165    170    175
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
180    185    190
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
195    200    205
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
210    215    220
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
225    230    235    240
Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
245    250    255
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
260    265    270
Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
275    280    285
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
290    295    300

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Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
305 310 315 320

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
325 330 335

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
340 345 350

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
355 360 365

<210> SEQ ID NO 248

<211> LENGTH: 1098

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: IL-2 qm-Fc knob (LALA P329G) (IL-2 N-terminal)

<400> SEQUENCE: 248

```
gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat      60
ctgcagatga tctgaacgg catcaacaac tacaagaacc ccaagctgac ccggtgctg      120
accgccaagt tcgccatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa      180
gaggaaactga agcctctgga agagggtgctg aacggcgccc agtccaagaa cttccacctg      240
aggcctcggg acctgatctc caacatcaac gtgatcgtgc tggaactgaa gggctccgag      300
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg      360
tggatcacct tcgcccagtc catcatctcc accctgacct ccggtggtgg cggatccgac      420
aaaactcaca catgccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc      480
ctcttcccc caaaacccaa ggacacctc atgatctccc ggacctctga ggtcacatgc      540
gtggtggtgg acgtgagcca cgaagacct gaggtcaagt tcaactggta cgtggacggc      600
gtggagggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      660
gtggtcagcg tcctcacctg cctgcaccag gactggctga atggcaagga gtacaagtgc      720
aaggctctcca acaaaacct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg      780
cagccccgag aaccacaggt gtacacctg ccccatgcc gggatgagct gaccaagaac      840
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatgc cgtggagtgg      900
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac      960
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcagggggaa      1020
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagctc      1080
tccctgtctc cgggtaaa                                1098
```

<210> SEQ ID NO 249

<211> LENGTH: 384

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: IL-7-Fc knob (LALA P329G) (IL-7 N-terminal)

<400> SEQUENCE: 249

Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu  
1 5 10 15

Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly Ser  
20 25 30

Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp  
35 40 45

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Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg  
50 55 60

Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu Leu  
65 70 75 80

Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val  
85 90 95

Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys Ser  
100 105 110

Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu  
115 120 125

Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys  
130 135 140

Ile Leu Met Gly Thr Lys Glu His Gly Gly Gly Ser Asp Lys Thr  
145 150 155 160

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser  
165 170 175

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
180 185 190

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
195 200 205

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
210 215 220

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val  
225 230 235 240

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
245 250 255

Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr  
260 265 270

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
275 280 285

Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys  
290 295 300

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
305 310 315 320

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
325 330 335

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
340 345 350

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
355 360 365

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
370 375 380

&lt;210&gt; SEQ ID NO 250

&lt;211&gt; LENGTH: 1152

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IL-7-Fc knob (LALA P329G) (IL-7 N-terminal)

&lt;400&gt; SEQUENCE: 250

gattgtgata ttgaaggtaa agatggcaaa caatatgaga gtgttctaataa ggtagcagcatc 60

gatcaattat tggacagcat gaaagaaatt ggtagcaatt gcctgaataa tgaatttaac 120

ttttttaaaa gacatatctg tgatgctaataa aaggaaggta tgtttttatt ccgtgctgct 180

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cgcaagttga ggcaatttct taaaatgaat agcactgggtg attttgatct ccacttatta 240
aaagtttcag aaggcacaac aatactgttg aactgcactg gccagggttaa aggaagaaaa 300
ccagctgccc tgggtgaagc ccaaccaaca aagagtttgg aagaaaataa atctttaaag 360
gaacagaaaa aactgaatga ctgtgttttc ctaaagagac tattacaaga gataaaaact 420
tgttggaata aaattttgat gggcactaaa gaacacgggtg gtggcggatc cgacaaaact 480
cacacatgcc caccgtgccc agcacctgaa gctgcagggg gaccgtcagt ctctctcttc 540
ccccaaaaac ccaaggacac cctcatgac tcccggaacc ctgaggtcac atgctgggtg 600
gtggacgtga gccacgaaga cctgaggtc aagttcaact ggtacgtgga cggcgtggag 660
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc 720
agcgtctca cgtctctga ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc 780
tccaaaaaag ccctcggcgc ccccatcgag aaaaccatct ccaaagccaa agggcagccc 840
cgagaaccac aggtgtacac cctgccccca tgccgggatg agctgaccaa gaaccaggtc 900
agcctgtggt gcctgttcaa aggcctctat cccagcgaca tcgccgtgga gtgggagagc 960
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc 1020
ttctctctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc 1080
tcatgctccg tgatgcata ggctctgcac aaccactaca cgcagaagag cctctccctg 1140
tctccgggta aa 1152

```

&lt;210&gt; SEQ ID NO 251

&lt;211&gt; LENGTH: 398

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IFN-a-Fc knob (LALA P329G) (IFN-a N-terminal)

&lt;400&gt; SEQUENCE: 251

```

Ser Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
1             5             10             15

Met Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys
20            25            30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
35            40            45

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
50            55            60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
65            70            75            80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
85            90            95

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
100           105           110

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
115           120           125

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130           135           140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145           150           155           160

Ser Leu Arg Ser Lys Glu Gly Gly Gly Ser Asp Lys Thr His Thr
165           170           175

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe

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180	185	190
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro		
195	200	205
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val		
210	215	220
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr		
225	230	235
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val		
245	250	255
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys		
260	265	270
Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser		
275	280	285
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		
290	295	300
Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val		
305	310	315
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
325	330	335
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp		
340	345	350
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp		
355	360	365
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His		
370	375	380
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		
385	390	395

&lt;210&gt; SEQ ID NO 252

&lt;211&gt; LENGTH: 1194

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: IFN-a-Fc knob (LALA P329G) (IFN-a N-terminal)

&lt;400&gt; SEQUENCE: 252

```

agctgtgacc tgcctcagac acacagcctg ggcagccggc ggaccctgat gctgctggcc      60
cagatgcgga agatcagcct gtccagctgc ctgaaggacc ggcacgactt cggttccct      120
caggaagagt tcggcaacca gttccagaag gccgagacaa tccccgtgct gcacgagatg      180
atccagcaga ttttcaacct gtccagcacc aaggacagca gcgcgcctg ggacgagaca      240
ctgctggaca agttctacac cgagctgtac cagcagctga acgacctgga agcctgctg      300
atccagggcg tgggctgtac cgagacaccc ctgatgaagg aagatagcat cctggccgtg      360
cggaagtatt tccagcggat caccctgtac ctgaaagaga agaagtacag cccctgcgcc      420
tgggaggtcg tgcggggcga gatcatgcgg agcttcagcc tgagcaccaa cctgcaggaa      480
agcctgcgga gcaaaagagg tggtggcgga tccgacaaaa ctcacacatg cccaccgtgc      540
ccagcacctg aagctgcagg gggaccgtca gtcttctctt tcccccaaa acccaaggac      600
accctcatga tctcccgga ccttgaggtc acatgcgttg tgggtggact gagccacgaa      660
gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca      720
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtctt caccgtctg      780
caccaggact ggctgaatgg caaggagtac aagtgaagg tctccaacaa agccctcggc      840

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gcccccatcg agaaaacccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac   900
accctgcccc catgccggga tgagctgacc aagaaccagg tcagcctgtg gtgcctggtc   960
aaaggcttct atcccagcga catgcgcgtg gagtgggaga gcaatgggca gccggagAAC   1020
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttctc ctacagcaag   1080
ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgtct cgtgatgcat   1140
gaggctctgc acaaccacta cagcagaag agcctctccc tgtctccggg taaa         1194

```

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<210> SEQ ID NO 253
<211> LENGTH: 593
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 28H1 Fab HC-Fc (LALA P329G)-IL-2 qm

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<400> SEQUENCE: 253

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50          55          60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65          70          75          80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85          90          95
Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100         105         110
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115         120         125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130         135         140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145         150         155         160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165         170         175
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180         185         190
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195         200         205
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210         215         220
Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
225         230         235         240
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245         250         255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260         265         270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275         280         285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290         295         300

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Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	
305					310					315					320	
Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	
				325					330					335		
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	
			340					345					350			
Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	
		355					360					365				
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	
	370					375					380					
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	
385					390					395					400	
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	
				405					410					415		
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	
			420					425					430			
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	
		435					440					445				
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Pro	Ala	Ser	
	450					455					460					
Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	
465					470					475					480	
Leu	Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	
			485					490						495		
Thr	Arg	Met	Leu	Thr	Ala	Lys	Phe	Ala	Met	Pro	Lys	Lys	Ala	Thr	Glu	
		500						505					510			
Leu	Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	
		515					520					525				
Val	Leu	Asn	Gly	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	
	530					535					540					
Leu	Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	
545					550					555					560	
Thr	Thr	Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr	Ile	Val	Glu	
			565						570					575		
Phe	Leu	Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser	Ile	Ile	Ser	Thr	Leu	
		580					585						590			

Thr

&lt;210&gt; SEQ ID NO 254

&lt;211&gt; LENGTH: 1779

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1 Fab HC-Fc (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 254

gaagtcagc	tgctggaatc	cggcgaggc	ctggtgcagc	ctggcggatc	tctgagactg	60
tctgcgcgcg	cctccggett	caccttctcc	tcccacgcc	tgtcctgggt	ccgacaggct	120
cctggcaaag	gcctggaatg	ggtgtccgcc	atctgggcct	ccggcgagca	gtactacgcc	180
gactctgtga	agggccggtt	caccatctcc	cgggacaact	ccaagaacac	cctgtacctg	240
cagatgaact	ccctgcgggc	cgaggacacc	gccgtgtact	actgtgccaa	gggctggctg	300
ggcaacttcg	actactgggg	acagggcacc	ctggtcaccg	tgtccagcgc	tagcaccaag	360
ggcccatcgg	tcttccccct	ggcaccctcc	tccaagagca	cctctggggg	cacagcggcc	420

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ctgggctgcc tggtaagga ctacttcccc gaaccgggtga cgggtgctgtg gaactcaggc 480
gccctgacca gcggcgtgca cacttccccg gctgtcctac agtcctcagg actctactcc 540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta catctgcaac 600
gtgaatcaca agcccagcaa caccaagggtg gacaagaaag ttgagcccaa atcttgtgac 660
aaaactcaca catgccccacc gtgccagca cctgaagctg cagggggacc gtcagtcttc 720
ctcttcccc caaaacccaa ggacacccctc atgatctccc ggacccctga ggtcacatgc 780
gtgggtgggtg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 900
gtggtcagcg tcctcacgt cctgcaccag gactggctga atggcaagga gtacaagtgc 960
aaggctctcca acaaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg 1020
cagccccgag aaccacaggt gtacaccctg ccccatccc gggatgagct gaccaagaac 1080
caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatgc cgtggagtgg 1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac 1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggctcc 1380
gcacctgcct caagtcttac aaagaaaaca cagctacaac tggagcattt actgctggat 1440
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 1500
acagccaagt ttgccatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 1560
gaagaactca aacctctgga ggaagtgcta aatggcgctc aaagcaaaaa ctttcaacta 1620
agaccagggt acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 1680
acaacattca tgtgtgaata tgetgatgag acagcaacca ttgtagaatt tctgaacaga 1740
tggattacct ttgcccagg catcatctca acactgact 1779

```

&lt;210&gt; SEQ ID NO 255

&lt;211&gt; LENGTH: 473

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Murine IL-2R-beta-Fc(hole) fusion protein

&lt;400&gt; SEQUENCE: 255

```

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp
1           5           10           15
Phe Pro Leu Leu Leu Trp Phe Pro Gly Ala Arg Cys Ala Val Lys
20           25           30
Asn Cys Ser His Leu Glu Cys Phe Tyr Asn Ser Arg Ala Asn Val Ser
35           40           45
Cys Met Trp Ser His Glu Glu Ala Leu Asn Val Thr Thr Cys His Val
50           55           60
His Ala Lys Ser Asn Leu Arg His Trp Asn Lys Thr Cys Glu Leu Thr
65           70           75           80
Leu Val Arg Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ser Phe
85           90           95
Pro Glu Ser Gln Ser Leu Thr Ser Val Asp Leu Leu Asp Ile Asn Val
100          105          110
Val Cys Trp Glu Glu Lys Gly Trp Arg Arg Val Lys Thr Cys Asp Phe

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115	120	125
His Pro Phe Asp Asn Leu Arg	Leu Val Ala Pro	His Ser Leu Gln Val
130	135	140
Leu His Ile Asp Thr Gln Arg Cys Asn Ile Ser Trp Lys Val Ser Gln		
145	150	155 160
Val Ser His Tyr Ile Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg Arg		
165	170	175
Leu Leu Gly His Ser Trp Glu Asp Ala Ser Val Leu Ser Leu Lys Gln		
180	185	190
Arg Gln Gln Trp Leu Phe Leu Glu Met Leu Ile Pro Ser Thr Ser Tyr		
195	200	205
Glu Val Gln Val Arg Val Lys Ala Gln Arg Asn Asn Thr Gly Thr Trp		
210	215	220
Ser Pro Trp Ser Gln Pro Leu Thr Phe Arg Thr Arg Pro Ala Asp Pro		
225	230	235 240
Met Lys Glu Gly Ala Gln Asp Lys Thr His Thr Cys Pro Pro Cys Pro		
245	250	255
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys		
260	265	270
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val		
275	280	285
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr		
290	295	300
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu		
305	310	315 320
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His		
325	330	335
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys		
340	345	350
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln		
355	360	365
Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu		
370	375	380
Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro		
385	390	395 400
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn		
405	410	415
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu		
420	425	430
Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val		
435	440	445
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln		
450	455	460
Lys Ser Leu Ser Leu Ser Pro Gly Lys		
465	470	

&lt;210&gt; SEQ ID NO 256

&lt;211&gt; LENGTH: 1422

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Murine IL-2R-beta-Fc(hole) fusion protein

&lt;400&gt; SEQUENCE: 256

atggacatga gggccccgc tcagctcctg ggcctcctgc tgctctgggt cccctcctg

60

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ctgctctggt tcccaggtgc caggtgtgca gtgaaaaact gttcccatct tgaatgcttc 120
tacaactcaa gagccaatgt ctcttgcatg tggagccatg aagaggtctct gaatgtcaca 180
acctgccacg tccatgccaa gtcgaacctg cgacactgga acaaaacctg tgagctaact 240
cttgtgaggc aggcatcctg ggctgcaac ctgacccctg ggtcggtccc agagtcccag 300
tcaactgacct ccgtggacct ccttgacata aatgtggtgt gctgggaaga gaagggttgg 360
cgtagggtaa agacctgcca cttccatccc ttgacaacc ttgcctgggt ggcccctcat 420
tccctccaag ttctgcacat tgatacccag agatgtaaca taagctggaa ggtctcccag 480
gtctctcact acattgaacc atacttgaa ttgaggccc gtagacgtct tctgggccac 540
agctgggagg atgcatccgt attaacctc aagcagagac agcagtggct cttcttgagg 600
atgctgatcc ctagtacctc atatgaggtc caggtgaggg tcaaagctca acgaaacaat 660
accgggacct ggagtcctcg gagccagccc ctgaccttcc ggacaaggcc agcagatccc 720
atgaaggagg gagctcagga caaaactcac acatgccccc cgtgcccagc acctgaactc 780
ctggggggac cgtcagtcct cctcttcccc ccaaaaccca aggacacct catgatctcc 840
cggacctctg aggtcacatg cgtggtggtg gacgtgagcc acgaagacct tgaggtaag 900
ttcaactggt acgtggacgg cgtggagggtg cataatgcca agacaaagcc gcgggaggag 960
cagtacaaca gcacgtaccg tgtggtcagc gtcctcacgg tcctgcacca ggactggctg 1020
aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1080
accatctcca aagccaaagg gcagccccga gaaccacagg tgtgcacct gcccccattc 1140
cgggatgagc tgaccaagaa ccaggtcagc ctctcgtgcy cagtcaaagg cttctatccc 1200
agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1260
cctcccgtgc tggactccga cggtcctctc ttcctcgtga gcaagctcac cgtggacaag 1320
agcaggtggc agcaggggaa cgtctcttca tgctcgtga tgcatgaggc tctgcacaac 1380
cactacacgc agaagagcct ctcccgtgtc ccgggtaaat ga 1422

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&lt;210&gt; SEQ ID NO 257

&lt;211&gt; LENGTH: 500

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Murine IL-2R-gamma-Fc(knob) fusion protein

&lt;400&gt; SEQUENCE: 257

```

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp
1           5           10           15
Phe Pro Leu Leu Leu Trp Phe Pro Gly Ala Arg Cys Trp Ser Ser
20          25          30
Lys Val Leu Met Ser Ser Ala Asn Glu Asp Ile Lys Ala Asp Leu Ile
35          40          45
Leu Thr Ser Thr Ala Pro Glu His Leu Ser Ala Pro Thr Leu Pro Leu
50          55          60
Pro Glu Val Gln Cys Phe Val Phe Asn Ile Glu Tyr Met Asn Cys Thr
65          70          75          80
Trp Asn Ser Ser Ser Glu Pro Gln Ala Thr Asn Leu Thr Leu His Tyr
85          90          95
Arg Tyr Lys Val Ser Asp Asn Asn Thr Phe Gln Glu Cys Ser His Tyr
100         105         110
Leu Phe Ser Lys Glu Ile Thr Ser Gly Cys Gln Ile Gln Lys Glu Asp

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115	120	125
Ile Gln Leu Tyr Gln Thr	Phe Val Val Gln Leu	Gln Asp Pro Gln Lys
130	135	140
Pro Gln Arg Arg Ala Val	Gln Lys Leu Asn Leu	Gln Asn Leu Val Ile
145	150	155
Pro Arg Ala Pro Glu Asn Leu	Thr Leu Ser Asn Leu	Ser Glu Ser Gln
	165	170
Leu Glu Leu Arg Trp Lys	Ser Arg His Ile Lys	Glu Arg Cys Leu Gln
	180	185
Tyr Leu Val Gln Tyr Arg	Ser Asn Arg Asp Arg	Ser Trp Thr Glu Leu
	195	200
Ile Val Asn His Glu Pro	Arg Phe Ser Leu Pro	Ser Val Asp Glu Leu
	210	215
Lys Arg Tyr Thr Phe Arg	Val Arg Ser Arg Tyr	Asn Pro Ile Cys Gly
	225	230
Ser Ser Gln Gln Trp Ser	Lys Trp Ser Gln Pro	Val His Trp Gly Ser
	245	250
His Thr Val Glu Glu Asn	Pro Ser Leu Phe Ala	Leu Glu Ala Gly Ala
	260	265
Gln Asp Lys Thr His Thr	Cys Pro Pro Cys Pro	Ala Pro Glu Leu Leu
	275	280
Gly Gly Pro Ser Val Phe	Leu Phe Pro Pro Lys	Pro Lys Asp Thr Leu
	290	295
Met Ile Ser Arg Thr Pro	Glu Val Thr Cys Val	Val Val Asp Val Ser
	305	310
His Glu Asp Pro Glu Val	Lys Phe Asn Trp Tyr	Val Asp Gly Val Glu
	325	330
Val His Asn Ala Lys Thr	Lys Pro Arg Glu Glu	Gln Tyr Asn Ser Thr
	340	345
Tyr Arg Val Val Ser Val	Leu Thr Val Leu His	Gln Asp Trp Leu Asn
	355	360
Gly Lys Glu Tyr Lys Cys	Lys Val Ser Asn Lys	Ala Leu Pro Ala Pro
	370	375
Ile Glu Lys Thr Ile Ser	Lys Ala Lys Gly Gln	Pro Arg Glu Pro Gln
	385	390
Val Tyr Thr Leu Pro Pro	Cys Arg Asp Glu Leu	Thr Lys Asn Gln Val
	405	410
Ser Leu Trp Cys Leu Val	Lys Gly Phe Tyr Pro	Ser Asp Ile Ala Val
	420	425
Glu Trp Glu Ser Asn Gly	Gln Pro Glu Asn Asn	Tyr Lys Thr Thr Pro
	435	440
Pro Val Leu Asp Ser Asp	Gly Ser Phe Phe Leu	Tyr Ser Lys Leu Thr
	450	455
Val Asp Lys Ser Arg Trp	Gln Gln Gly Asn Val	Phe Ser Cys Ser Val
	465	470
Met His Glu Ala Leu His	Asn His Tyr Thr	Gln Lys Ser Leu Ser
	485	490
Ser Pro Gly Lys		495
		500

&lt;210&gt; SEQ ID NO 258

&lt;211&gt; LENGTH: 1503

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

-continued

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Murine IL-2R-gamma-Fc(knob) fusion protein

&lt;400&gt; SEQUENCE: 258

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atggacatga gggccccgc tcagctcctg ggcctcctgc tgctctgggt cccctcctg      60
ctgctctggg tcccagggtg cagggtgttg agttccaagg tcctcatgtc cagtgcgaat     120
gaagacatca aagctgattt gatcctgact tctacagccc ctgaacacct cagtgtcct      180
actctgcccc ttccagaggt tcagtgtttt gtgttcaaca tagagtacat gaattgcact     240
tggaatatga gttctgagcc tcaggcaacc aacctcacgc tgcactatag gtacaaggta     300
tctgataata atacattcca ggagtgcagt cactatttgt tctccaaaga gattacttct     360
ggctgtcaga taaaaaaga agatatccag ctctaccaga catttgttgt ccagctccag     420
gacccccaga aacccagag gcgagctgta cagaagctaa acctacagaa tcttgtgatc     480
ccacgggctc cagaaaatct aacctcagc aatctgagtg aatcccagct agagctgaga     540
tggaanaagc gacatattaa agaacgctgt ttacaatact tgggtgcagta ccggagcaac     600
agagatcgaa gctggacgga actaatagtg aatcatgaac ctagattctc cctgcctagt     660
gtggatgagc tgaaacggta cacatttcgg gttcggagcc gctataaccc aatctgtgga     720
agtttctaac agtggagtaa atggagccag cctgtccact gggggagtc tactgtagag     780
gagaatcctt ccttgtttgc actggaagct ggagctcagg acaaaaactc cacatgccca     840
ccgtgcccag cacctgaact cctgggggga ccgtcagttt tcctcttccc cccaaaaccc     900
aaggacaccc tcattgatct ccggacccct gaggtcacat gcgtgggtgt ggacgtgagc     960
cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc    1020
aagacaaaag cgcgaggagga gcagtacaac agcacgtacc gtgtgggtcag cgtcctcacc    1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc    1140
ctcccagccc ccattcgagaa aacctctccc aaagccaaag ggcagccccc agaaccacag    1200
gtgtacaccc tgcccccatg ccgggatgag ctgaccaaga accaggtcag cctgtgggtg    1260
ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccc    1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac    1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg    1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa    1500
tga

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&lt;210&gt; SEQ ID NO 259

&lt;211&gt; LENGTH: 213

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Murine IL-2R alpha subunit + Avi-tag + His-tag

&lt;400&gt; SEQUENCE: 259

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1           5           10          15

Val His Ser Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro Asn Ala
          20          25          30

Thr Phe Lys Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu
          35          40          45

Cys Lys Arg Gly Phe Arg Arg Leu Lys Glu Leu Val Tyr Met Arg Cys
          50          55          60

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Leu Gly Asn Ser Trp Ser Ser Asn Cys Gln Cys Thr Ser Asn Ser His  
 65 70 75 80  
 Asp Lys Ser Arg Lys Gln Val Thr Ala Gln Leu Glu His Gln Lys Glu  
 85 90 95  
 Gln Gln Thr Thr Thr Asp Met Gln Lys Pro Thr Gln Ser Met His Gln  
 100 105 110  
 Glu Asn Leu Thr Gly His Cys Arg Glu Pro Pro Pro Trp Lys His Glu  
 115 120 125  
 Asp Ser Lys Arg Ile Tyr His Phe Val Glu Gly Gln Ser Val His Tyr  
 130 135 140  
 Glu Cys Ile Pro Gly Tyr Lys Ala Leu Gln Arg Gly Pro Ala Ile Ser  
 145 150 155 160  
 Ile Cys Lys Met Lys Cys Gly Lys Thr Gly Trp Thr Gln Pro Gln Leu  
 165 170 175  
 Thr Cys Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly Ser Gly Leu Asn  
 180 185 190  
 Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu Ala Arg Ala His  
 195 200 205  
 His His His His His  
 210

<210> SEQ ID NO 260  
 <211> LENGTH: 642  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Murine IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 260

```

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccgaa    60
ctgtgtctgt atgaccaccc cgaggctccc aatgccacat tcaaagccct ctctacaag    120
aacggcacca tcctaaactg tgaatgcaag agaggtttcc gaagactaaa ggaattggtc    180
tatatgcgtt gcttaggaaa ctcttgaggc agcaactgcc agtgcaccag caactcccat    240
gacaaatcga gaaagcaagt tacagctcaa cttgaacacc agaaagagca acaaaccaca    300
acagacatgc agaagccaac acagtctatg caccaagaga accttacagg tctactgcagg    360
gagccacctc cttggaaaca tgaagattcc aagagaatct atcatttcgt ggaaggacag    420
agtgttcaact acgagtgtat tccgggatac aaggctctac agagaggtcc tgctattagc    480
atctgcaaga tgaagtgtgg gaaaacgggg tggactcagc cccagctcac atgtgtcgac    540
gaacagttat attttcaggg cggtcaggc ctgaacgaca tcttcgaggg ccagaagatc    600
gagtggcacg aggtctgagc tcaccacccat caccatcact ga                      642
  
```

<210> SEQ ID NO 261  
 <211> LENGTH: 480  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Cynomolgous IL-2R-beta-Fc(knob) fusion  
 protein + Avi-tag

<400> SEQUENCE: 261

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15  
 Val His Ser Ala Val Asn Gly Thr Ser Arg Phe Thr Cys Phe Tyr Asn  
 20 25 30

Ser 35	Arg	Ala	Asn	Ile	Ser	Cys	Val	Trp	Ser	Gln	Asp	Gly	Ala	Leu	Gln
Asp 50	Thr	Ser	Cys	Gln	Val	His	Ala	Trp	Pro	Asp	Arg	Arg	Arg	Trp	Asn
Gln 65	Thr	Cys	Glu	Leu	Leu	Pro	Val	Ser	Gln	Ala	Ser	Trp	Ala	Cys	Asn
Leu	Ile	Leu	Gly	Thr	Pro	Asp	Ser	Gln	Lys	Leu	Thr	Ala	Val	Asp	Ile
Val	Thr	Leu	Arg	Val	Met	Cys	Arg	Glu	Gly	Val	Arg	Trp	Arg	Met	Met
Ala	Ile	Gln	Asp	Phe	Lys	Pro	Phe	Glu	Asn	Leu	Arg	Leu	Met	Ala	Pro
Ile	Ser	Leu	Gln	Val	Val	His	Val	Glu	Thr	His	Arg	Cys	Asn	Ile	Ser
Trp 145	Lys	Ile	Ser	Gln	Ala	Ser	His	Tyr	Phe	Glu	Arg	His	Leu	Glu	Phe
Glu	Ala	Arg	Thr	Leu	Ser	Pro	Gly	His	Thr	Trp	Glu	Glu	Ala	Pro	Leu
Met	Thr	Leu	Lys	Gln	Lys	Gln	Glu	Trp	Ile	Cys	Leu	Glu	Thr	Leu	Thr
Pro	Asp	Thr	Gln	Tyr	Glu	Phe	Gln	Val	Arg	Val	Lys	Pro	Leu	Gln	Gly
Glu	Phe	Thr	Thr	Trp	Ser	Pro	Trp	Ser	Gln	Pro	Leu	Ala	Phe	Arg	Thr
Lys 225	Pro	Ala	Ala	Leu	Gly	Lys	Asp	Thr	Gly	Ala	Gln	Asp	Lys	Thr	His
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
Thr 305	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu
Val 385	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Ser

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450	455	460	
Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu			
465	470	475	480

<210> SEQ ID NO 262  
 <211> LENGTH: 1443  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Cynomolgous IL-2R-beta-Fc(knob) fusion protein + Avi-tag

<400> SEQUENCE: 262

```

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccgcg      60
gtcaacggca cttcccgggt cacatgcttc tacaactcga gagccaacat ctctgtgttc      120
tggagccaag atggggctct gcaggacact tctgccaag tccacgcctg gccggacaga      180
cggcgggtgga accaaacctg tgagctgctc cctgtgagtc aagcatcctg ggctgcaac      240
ctgatcctcg gaaccccgaga ttctcagaaa ctgaccgcag tggatatcgt caccctgagg      300
gtgatgtgcc gtgaaggggt gcgatggagg atgatggcca tccaggactt caaacccctt      360
gagaaccttc gcctgatggc ccccatctcc ctccaagtcg tccacgtgga gaccacaga      420
tgcaacataa gctggaaaat ctccaagcc tcccactact ttgaaagaca cctggagttt      480
gaggcccgga cgctgtcccc aggccacacc tgggaggagg ccccccctgat gaccctcaag      540
cagaagcagg aatggatctg cctggagacg ctcacccag acaccagta tgagtttcag      600
gtgcgggtca agcctctgca aggcgagttc acgacctgga gccctggag ccagccctg      660
gccttcagga caaagcctgc agcccttggg aaggacaccg gagctcagga caaaactcac      720
acatgcccac cgtgcccagc acctgaactc ctggggggac cgtcagtctt cctcttcccc      780
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg      840
gacgtgagcc acgaagacc tgaggtaag ttcaactggt acgtggacgg cgtggagggtg      900
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc      960
gtcctcaccg tcttcacca ggactggctg aatggcaagg agtacaagt caaggtctcc      1020
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga      1080
gaaccacagg tgtacacct gcccccatgc cgggatgagc tgaccaagaa ccaggtcagc      1140
ctgtggtgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat      1200
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggtccttc      1260
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca      1320
tgctccgtga tgcattgggc tctgcacaac cactacacgc agaagagcct ctccctgtct      1380
ccgggtaaat ccggaggcct gaacgacatc ttcgaggccc agaagattga atggcacgag      1440
tga

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tga 1443

<210> SEQ ID NO 263  
 <211> LENGTH: 489  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Cynomolgous IL-2R-gamma-Fc(hole) fusion protein

<400> SEQUENCE: 263

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val	His	Ser	Leu	Asn	Thr	Thr	Ile	Leu	Thr	Pro	Asn	Gly	Asn	Glu	Asp
			20					25					30		
Ala	Thr	Thr	Asp	Phe	Phe	Leu	Thr	Ser	Met	Pro	Thr	Asp	Ser	Leu	Ser
		35					40					45			
Val	Ser	Thr	Leu	Pro	Leu	Pro	Glu	Val	Gln	Cys	Phe	Val	Phe	Asn	Val
	50					55				60					
Glu	Tyr	Met	Asn	Cys	Thr	Trp	Asn	Ser	Ser	Ser	Glu	Pro	Gln	Pro	Thr
65					70					75					80
Asn	Leu	Thr	Leu	His	Tyr	Trp	Tyr	Lys	Asn	Ser	Asp	Asn	Asp	Lys	Val
				85					90					95	
Gln	Lys	Cys	Ser	His	Tyr	Leu	Phe	Ser	Glu	Glu	Ile	Thr	Ser	Gly	Cys
			100					105					110		
Gln	Leu	Gln	Lys	Lys	Glu	Ile	His	Leu	Tyr	Gln	Thr	Phe	Val	Val	Gln
		115					120					125			
Leu	Gln	Asp	Pro	Arg	Glu	Pro	Arg	Arg	Gln	Ala	Thr	Gln	Met	Leu	Lys
	130					135					140				
Leu	Gln	Asn	Leu	Val	Ile	Pro	Trp	Ala	Pro	Glu	Asn	Leu	Thr	Leu	Arg
145					150					155					160
Lys	Leu	Ser	Glu	Ser	Gln	Leu	Glu	Leu	Asn	Trp	Asn	Asn	Arg	Phe	Leu
			165						170					175	
Asn	His	Cys	Leu	Glu	His	Leu	Val	Gln	Tyr	Arg	Thr	Asp	Trp	Asp	His
			180					185					190		
Ser	Trp	Thr	Glu	Gln	Ser	Val	Asp	Tyr	Arg	His	Lys	Phe	Ser	Leu	Pro
		195					200					205			
Ser	Val	Asp	Gly	Gln	Lys	Arg	Tyr	Thr	Phe	Arg	Val	Arg	Ser	Arg	Phe
	210					215					220				
Asn	Pro	Leu	Cys	Gly	Ser	Ala	Gln	His	Trp	Ser	Glu	Trp	Ser	His	Pro
225					230					235					240
Ile	His	Trp	Gly	Ser	Asn	Ser	Ser	Lys	Glu	Asn	Pro	Phe	Leu	Phe	Ala
			245						250					255	
Leu	Glu	Ala	Gly	Ala	Gln	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro
			260					265					270		
Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys
		275					280					285			
Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val
	290					295					300				
Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr
305					310					315					320
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu
			325						330					335	
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His
			340					345					350		
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys
		355					360					365			
Ala	Leu	Gly	Ala	Pro	Ile										

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435	440	445	
Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val			
450	455	460	
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln			
465	470	475	480
Lys Ser Leu Ser Leu Ser Pro Gly Lys			
485			

<210> SEQ ID NO 264  
 <211> LENGTH: 1470  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Cynomolgous IL-2R-gamma-Fc(hole) fusion protein

<400> SEQUENCE: 264

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atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccctg      60
aacacgacaa ttctgacgcc caatgggaat gaagacgcca caactgattt cttcctgacc      120
tctatgccca ctgactccct cagtgtttcc actctgcccc tcccagaggt tcagtgtttt      180
gtgttcaatg tcgagtacat gaattgcact tggaacagca gctctgagcc ccagcctacc      240
aacctcactc tgcattattg gtacaagaat tcggataatg ataaagtcca gaagtgcagc      300
cactatctat tctctgaaga aatcacttct ggctgtcagt tgcaaaaaaa ggagatccac      360
ctctacaaaa cgtttgttgt tcagctccag gacccacggg aaccaggag acagggccaca      420
cagatgctaa aactgcagaa tctggtgata ccttgggctc cggagaacct aacacttcgc      480
aaactgagtg aatcccagct agaactgaac tggaacaaca gattcttgaa ccactgtttg      540
gagcacttgg tgcagtaccg gactgactgg gaccacagct ggactgaaca atcagtggat      600
tatagacata agttctcctt gcctagtgtg gatgggcaga aacgctacac gtttcgtgtc      660
cggagccgct ttaaccact ctgtggaagt gctcagcatt ggagtgaatg gagccacca      720
atccactggg ggagcaatag ttcaaaagag aatcctttcc tgtttgcatt ggaagccgga      780
gctcaggaca aaactcacac atgcccaccg tgcccagcac ctgaactcct ggggggaccg      840
tcagtcttcc tcttcccccc aaaacccaag gacacctca tgatctcccg gaccttgag      900
gtcacatgcg tgggtgtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac      960
gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc     1020
acgtaccgtg tggtcagcgt cctcacgctc ctgcaccagg actggctgaa tggcaaggag     1080
tacaagtgca aggtctccaa caaagccctc ggcgccccca tcgagaaaac catctccaaa     1140
gccaaggggc agccccgaga accacagggtg tgcaccctgc ccccatcccc ggatgagctg     1200
accaagaacc aggtcagcct ctctgtcgca gtcaaaaggct tctatcccag cgacatcgcc     1260
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgtg     1320
gactccgacg gctccttctt cctcgtgagc aagctcaccg tggacaagag cagggtggcag     1380
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag     1440
aagagcctct cctgtctctc gggtaaatga

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<210> SEQ ID NO 265  
 <211> LENGTH: 217  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Cynomolgous IL-2R alpha subunit +  
 Avi-tag + His-tag

-continued

&lt;400&gt; SEQUENCE: 265

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15  
 Glu Leu Cys Asp Asp Asp Pro Pro Lys Ile Thr His Ala Thr Phe Lys  
 20 25 30  
 Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg  
 35 40 45  
 Gly Phe Arg Arg Ile Lys Ser Gly Ser Pro Tyr Met Leu Cys Thr Gly  
 50 55 60  
 Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser  
 65 70 75 80  
 Ala Ala Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln  
 85 90 95  
 Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Gln Met Gln Leu Ala Asp  
 100 105 110  
 Gln Val Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn  
 115 120 125  
 Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Thr Val Tyr  
 130 135 140  
 Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu  
 145 150 155 160  
 Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln  
 165 170 175  
 Leu Ile Cys Thr Gly Glu Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly  
 180 185 190  
 Ser Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu  
 195 200 205  
 Ala Arg Ala His His His His His His  
 210 215

&lt;210&gt; SEQ ID NO 266

&lt;211&gt; LENGTH: 654

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Cynomolgous IL-2R alpha subunit +  
 Avi-tag + His-tag

&lt;400&gt; SEQUENCE: 266

atgggatgga gctgtatcat cctctctctg gtagcaacag ctaccggtga gctctgtgac 60  
 gatgaccgcg caaaaatcac acatgccaca ttcaaagcca tggcctacaa ggaaggaacc 120  
 atgttgaaact gtgaatgcaa gagaggtttc cgcagaataa aaagcgggtc accctatatg 180  
 ctctgtacag gaaactctag ccactcgtcc tgggacaacc aatgtcaatg cacaagctct 240  
 gctgctcgga acacaacaaa acaagtgaca cctcaacctg aagaacagaa agaaagaaaa 300  
 accacagaaa tgcaaagtca aatgcagctg gcggaccaag tgagccttcc aggtcactgc 360  
 aggggaacctc caccgtggga aaatgaagcc acagaaagaa tttatcattt cgtggtgggg 420  
 cagacggttt actaccagtg cgtccaggga tacagggtc tacacagagg tcctgtgag 480  
 agcgtctgca aaatgaccca cggaagaca agatggaccc agccccagct catatgcaca 540  
 ggtgaagtgc acgaacagtt atattttcag ggcggctcag gcctgaacga catcttcgag 600  
 gcccaaga tcgagtggca cgaggctcga gctcaccacc atcaccatca ctga 654

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<210> SEQ ID NO 267
<211> LENGTH: 474
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-10R1-Fc fusion + Avi-tag

<400> SEQUENCE: 267

His Gly Thr Glu Leu Pro Ser Pro Pro Ser Val Trp Phe Glu Ala Glu
1          5          10          15

Phe Phe His His Ile Leu His Trp Thr Pro Ile Pro Asn Gln Ser Glu
20          25          30

Ser Thr Cys Tyr Glu Val Ala Leu Leu Arg Tyr Gly Ile Glu Ser Trp
35          40          45

Asn Ser Ile Ser Asn Cys Ser Gln Thr Leu Ser Tyr Asp Leu Thr Ala
50          55          60

Val Thr Leu Asp Leu Tyr His Ser Asn Gly Tyr Arg Ala Arg Val Arg
65          70          75          80

Ala Val Asp Gly Ser Arg His Ser Asn Trp Thr Val Thr Asn Thr Arg
85          90          95

Phe Ser Val Asp Glu Val Thr Leu Thr Val Gly Ser Val Asn Leu Glu
100         105         110

Ile His Asn Gly Phe Ile Leu Gly Lys Ile Gln Leu Pro Arg Pro Lys
115         120         125

Met Ala Pro Ala Asn Asp Thr Tyr Glu Ser Ile Phe Ser His Phe Arg
130         135         140

Glu Tyr Glu Ile Ala Ile Arg Lys Val Pro Gly Asn Phe Thr Phe Thr
145         150         155         160

His Lys Lys Val Lys His Glu Asn Phe Ser Leu Leu Thr Ser Gly Glu
165         170         175

Val Gly Glu Phe Cys Val Gln Val Lys Pro Ser Val Ala Ser Arg Ser
180         185         190

Asn Lys Gly Met Trp Ser Lys Glu Glu Cys Ile Ser Leu Thr Arg Gln
195         200         205

Tyr Phe Thr Val Thr Asn Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly
210         215         220

Ser Pro Lys Ser Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
225         230         235         240

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
245         250         255

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260         265         270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
275         280         285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290         295         300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305         310         315         320

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
325         330         335

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
340         345         350

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
355         360         365

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

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370	375	380	
Asp Ile Ala Val Glu Trp	Glu Ser Asn Gly Gln	Pro Glu Asn Asn Tyr	
385	390	395	400
Lys Thr Thr Pro Pro Val	Leu Asp Ser Asp Gly	Ser Phe Phe Leu Tyr	
	405	410	415
Ser Lys Leu Thr Val Asp	Lys Ser Arg Trp Gln	Gln Gly Asn Val Phe	
	420	425	430
Ser Cys Ser Val Met His	Glu Ala Leu His Asn	His Tyr Thr Gln Lys	
	435	440	445
Ser Leu Ser Leu Ser Pro	Gly Gly Gly Ser Gly	Gly Leu Asn Asp Ile	
	450	455	460
Phe Glu Ala Gln Lys Ile	Glu Trp His Glu		
465	470		
<210> SEQ ID NO 268			
<211> LENGTH: 1422			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Human IL-10R1-Fc fusion + Avi-tag			
<400> SEQUENCE: 268			
catgggacag agctgcccag ccctccgtct gtgtggtttg aagcagaatt ttccaccac			60
atcctccact ggacacccat cccaaatcag tctgaaagta cctgctatga agtggcactc			120
ctgaggtatg gaatagagtc ctggaactcc atctccaact gtagccagac cctgtcctat			180
gaccttaccg cagtgcctt ggacctgtac cacagcaatg gctaccgggc cagagtgcgg			240
gctgtggacg gcagccggca ctccaactgg accgtcacca acaccgcctt ctctgtggat			300
gaagtgactc tgacagtgg cagtgtgaac ctgagatcc acaatggctt catcctcggg			360
aagattcagc taccagggc caagatggcc cccgcaaag acacatatga aagcatcttc			420
agtcacttcc gagagtatga gattgccatt cgcaagggtc cgggaaactt cacgttcaca			480
cacaagaaag taaaacatga aaacttcagc ctcttaacct ctggagaagt gggagagttc			540
tgtgtccagg tgaaaccatc tgcgcttcc cgaagtaaca aggggatgtg gtctaaagag			600
gagtgcattc ccctcaccag gcagtatttc accgtgacca acgtcgacga acagtatat			660
tttcaggggc gctcacccaa atctgcagac aaaactcaca catgcccacc gtgccagca			720
cctgaactcc tggggggacc gtcagtcttc ctcttcccc caaaacccaa ggacaccctc			780
atgatctccc ggaccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct			840
gaggtaagt tcaactggta cgtggacggc gtggaggtgc ataagccaa gacaaagccg			900
cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag			960
gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct ccagcccc			1020
atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg			1080
cccccatccc gggatgagct gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc			1140
ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac			1200
aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctacag caagctcacc			1260
gtggacaaga gcagggtggc gcagggggaa gtcttctcat gctccgtgat gcatgaggct			1320
ctgcacaacc actacacgca gaagagcctc tcctgtctc cgggtggcgg gtcggagggc			1380
ctgaacgaca tcttcgaggc ccagaagatt gaatggcacg ag			1422

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<210> SEQ ID NO 269  
 <211> LENGTH: 593  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

<400> SEQUENCE: 269

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
          50          55          60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
          65          70          75          80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
          85          90          95

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
          100          105          110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
          115          120          125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
          130          135          140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
          145          150          155          160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
          165          170          175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
          180          185          190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
          195          200          205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
          210          215          220

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
          225          230          235          240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
          245          250          255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
          260          265          270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
          275          280          285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
          290          295          300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
          305          310          315          320

Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
          325          330          335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
          340          345          350

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
          355          360          365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
  
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370	375	380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp 385 390 395 400		
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp 405 410 415		
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His 420 425 430		
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly 435 440 445		
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala Ser 450 455 460		
Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp 465 470 475 480		
Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu 485 490 495		
Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu 500 505 510		
Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu 515 520 525		
Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp 530 535 540		
Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu 545 550 555 560		
Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu 565 570 575		
Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu 580 585 590		

Thr

&lt;210&gt; SEQ ID NO 270

&lt;211&gt; LENGTH: 1779

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

&lt;400&gt; SEQUENCE: 270

```

gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg      60
tctctgcgcg cctccggcct caccctctcc tcccacgcca tgtcctgggt ccgacaggct      120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct ccggcgagca gtactacgcc      180
gactctgtga agggccgggt caccatctcc cgggacaact ccaagaacac cctgtacctg      240
cagatgaact cctctcgggc cgaggacacc gccgtgtact actgtgccaa gggttggtg      300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag      360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc      420
ctgggctgcc tggtcaagga ctacttcccc gaaccgggtg cgggtgtcgtg gaactcaggc      480
gccctgacca gggcggtgca caccctcccg gctgtcttac agtcctcagg actctactcc      540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca ccagaccta catctgcaac      600
gtgaatcaca agcccagcaa caccaagggt gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780

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gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc   840
gtggagggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt   900
gtggtcagcg tcctcacogt cctgcaccag gactgggtga atggcaagga gtacaagtgc   960
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg  1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac  1080
caggtcagcc tgtggtgctt ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg  1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccggtgt ggactccgac  1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggc gcagggggaa  1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc  1320
tccttgtctc cgggtggcgg cggaggtctc ggaggcggag gttctggcgg aggtggctcc  1380
gcacctgcct caagtcttac aaagaaaaca cagctacaac tggagcattt actgctggat  1440
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc  1500
acagccaagt ttgccatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa  1560
gaagaactca aacctctgga ggaagtgcta aatggcgctc aaagcaaaaa ctttcactta  1620
agaccacggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa  1680
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga  1740
tggattacct ttgcccaaag catcatctca acactgact                               1779

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&lt;210&gt; SEQ ID NO 271

&lt;211&gt; LENGTH: 594

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

&lt;400&gt; SEQUENCE: 271

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5      10      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20     25     30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35     40     45
Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
50     55     60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65     70     75     80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85     90     95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100    105    110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115    120    125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130    135    140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145    150    155    160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165    170    175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180    185    190

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Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
	195						200					205			
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His
	210					215					220				
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val
225					230					235					240
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
				245					250					255	
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu
			260					265					270		
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
		275					280					285			
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
	290					295					300				
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
305					310					315					320
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile
				325					330					335	
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
			340					345					350		
Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu
		355					360					365			
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380				
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385					390					395					400
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
			405						410					415	
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
			420					425					430		
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly
	435					440						445			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Pro	Ala
	450					455					460				
Ser	Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu
465					470					475					480
Asp	Leu	Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys
			485						490					495	
Leu	Thr	Arg	Met	Leu	Thr	Ala	Lys	Phe	Ala	Met	Pro	Lys	Lys	Ala	Thr
			500					505				510			
Glu	Leu	Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu
		515				520						525			
Glu	Val	Leu	Asn	Gly	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg
	530					535					540				
Asp	Leu	Ile	Ser	Asn											

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<210> SEQ ID NO 272
<211> LENGTH: 1782
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

<400> SEQUENCE: 272

gagggtgcagc tgctcgaaag cggcggagga ctgggtgcagc ctggcggcag cctgagactg      60
tcttgcgccg ccagcggcgt caccctcagc agctacgccg tgagctgggt ccgccaggcc      120
cctggcaagg gactggaatg ggtgtccgcc atcatcggtt ctggcgccag caccctactac      180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac      240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggatgg      300
ttcggcgggt tcaactactg gggacagggc accctgggtc cagtgtccag cgctagcacc      360
aagggcccat cggctcttcc cctggcacc cctccaaga gcacctctgg gggcacagcg      420
gccctggggt gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgc gtggaactca      480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcttc aggactctac      540
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc      600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt      660
gacaaaactc acacatgcc cccgtgccca gcacctgaag ctgcaggggg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      780
tgcggtggtg tggacgtgag ccacgaagac cctgaggtea agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac      900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaagggtc ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag     1080
aaccaggtea gcctgtggtg cctggtcaaa ggctttctat ccagcgacat cgcogtggag     1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccggt gctggactcc     1200
gacggctcct tcttctctca cagcaagctc accgtggaca agagcagggt gcagcagggg     1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc     1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggttcttg cggaggtggc     1380
tccgcacctg cctcaagttc taaaaagaaa acacagctac aactggagca tttactgctg     1440
gatttacaga tgattttgaa tgaattaat aattacaaga atcccaact caccaggatg     1500
ctcacagcca agtttgccat gcccaagaag gccacagaac tgaacacatc tcagtgtcta     1560
gaagaagaac tcaaacctct ggaggaagtg ctaaatggcg ctcaaagcaa aaactttcac     1620
ttaagacca gggacttaat cagcaatatc aacgtaatag ttctggaact aaagggatct     1680
gaaacaacat tcattgtgtg atatgtgat gagacagcaa ccattgtaga atttctgaac     1740
agatggatta cctttgccca aagcatcatc tcaacactga ct                               1782

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<210> SEQ ID NO 273
<211> LENGTH: 594
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 wt

<400> SEQUENCE: 273

```

Glu 1	Val	Gln	Leu	Leu 5	Glu	Ser	Gly	Gly	Gly 10	Leu	Val	Gln	Pro	Gly 15	Gly
Ser	Leu	Arg	Leu 20	Ser	Cys	Ala	Ala	Ser 25	Gly	Phe	Thr	Phe	Ser 30	Ser	Tyr
Ala	Met	Ser 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
Ser	Ala 50	Ile	Ile	Gly	Ser	Gly 55	Ala	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val
Lys 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	Lys	Asn	Thr	Leu	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	Cys
Ala	Lys	Gly	Trp 100	Phe	Gly	Gly	Phe	Asn 105	Tyr	Trp	Gly	Gln 110	Gly	Thr	Leu
Val	Thr 115	Val	Ser	Ser	Ala	Ser	Thr 120	Lys	Gly	Pro	Ser	Val 125	Phe	Pro	Leu
Ala	Pro 130	Ser	Ser	Lys	Ser	Thr 135	Ser	Gly	Gly	Thr	Ala 140	Ala	Leu	Gly	Cys
Leu 145	Val	Lys	Asp	Tyr	Phe 150	Pro	Glu	Pro	Val	Thr 155	Val	Ser	Trp	Asn	Ser 160
Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr	Phe 170	Pro	Ala	Val	Leu	Gln 175	Ser
Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser	Val 185	Val	Thr	Val	Pro	Ser 190	Ser	Ser
Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile	Cys 200	Asn	Val	Asn	His	Lys 205	Pro	Ser	Asn
Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser	Cys 220	Asp	Lys	Thr	His
Thr 225	Cys	Pro	Pro	Cys	Pro 230	Ala	Pro	Glu	Ala	Ala 235	Gly	Gly	Pro	Ser	Val 240
Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp	Thr 250	Leu	Met	Ile	Ser	Arg 255	Thr
Pro	Glu	Val	Thr 260	Cys	Val	Val	Val	Asp 265	Val	Ser	His	Glu	Asp 270	Pro	Glu
Val	Lys 275	Phe	Asn	Trp	Tyr	Val	Asp 280	Gly	Val	Glu	Val	His 285	Asn	Ala	Lys
Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr	Tyr 300	Arg	Val	Val	Ser
Val 305	Leu	Thr	Val	Leu	His 310	Gln	Asp	Trp	Leu	Asn 315	Gly	Lys	Glu	Tyr	Lys 320
Cys	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Gly	Ala 330	Pro	Ile	Glu	Lys	Thr 335	Ile
Ser	Lys 340	Ala	Lys	Gly	Gln	Pro	Arg	Glu 345	Pro	Gln	Val	Tyr	Thr 350	Leu	Pro
Pro	Cys 355	Arg	Asp	Glu	Leu	Thr	Lys 360	Asn	Gln	Val	Ser	Leu 365	Trp	Cys	Leu
Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val	Glu 380	Trp	Glu	Ser	Asn
Gly 385	Gln	Pro	Glu	Asn	Asn 390	Tyr	Lys	Thr	Thr	Pro 395	Pro	Val	Leu	Asp	Ser 400
Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys	Leu 410	Thr	Val	Asp	Lys	Ser	Arg 415
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu

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420	425	430
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly		
435	440	445
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Thr		
450	455	460
Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu		
465	470	475
Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys		
485	490	495
Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr		
500	505	510
Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu		
515	520	525
Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg		
530	535	540
Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser		
545	550	555
Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val		
565	570	575
Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr		
580	585	590
Leu Thr		

&lt;210&gt; SEQ ID NO 274

&lt;211&gt; LENGTH: 1782

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 4B9 Fab HC-Fc knob (LALA P329G) -IL-2 wt

&lt;400&gt; SEQUENCE: 274

```

gaggtgcagc tgctcgaaag cggcggagga ctggtgcagc ctggcggcag cctgagactg      60
tcttgcgcgc ccagcggcgt caccctcagc agctacgccg tgagctgggt ccgccaggcc      120
cctggcaagg gactggaatg ggtgtccgcc atcatcggtc ctggcgccag caccctactac      180
gccgacagcg tgaagggcgc gtccaccatc agccgggaca acagcaagaa caccctgtac      240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggatgg      300
ttcggcgggt tcaactactg gggacagggc accctgggtc cagtgtccag cgctagcacc      360
aagggcccat cggtcttccc cctggcaccc tctccaaga gcacctctgg gggcacagcg      420
gccctggggt gcttgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca      480
ggcgccctga ccagcggcgt gcacacctc ccggctgtcc tacagtctc aggactctac      540
tccctcagca gcgtgggtgac cgtgcctccc agcagcttgg gcacccagac ctacatctgc      600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt      660
gacaaaactc acacatgccc accgtgccca gcacctgaag ctgcaggggg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      780
tgctgtggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac      900
cgtgtgtgtc gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag     1080

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aaccagggtca gcctgtggtg cctgggtcaaa ggctttctatc ccagcgacat cgccgtggag 1140
tgaggagagca atgggcagcc ggagaacaac tacaagacca cgctcccgt gctggactcc 1200
gacggctcct tcttcctcta cagcaagctc accgtggaca agagcagggtg gcagcagggg 1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
ctctccctgt ctccgggttg cgccggaggc tccggaggcg gaggttcttg aggcggaggc 1380
tccgcaccta cttcaagttc tacaagaaa acacagctac aactggagca tttactgctg 1440
gatttacaga tgattttgaa tggaattaat aattacaaga atcccaaact caccaggatg 1500
ctcacattta agttttacat gccaagaag gccacagaac tgaaacatct tcagtgtcta 1560
gaagaagaac tcaaacctct ggaggaagtg cttaaatttag ctcaaagcaa aaactttcac 1620
ttaagaccca gggacttaat cagcaatctc aacgtaatag ttctggaact aaagggatct 1680
gaaacaacat tcattgtgtga atatgctgat gagacagcaa ccattgtaga atttctgaac 1740
agatggatta cctttgcccc aagcatcatc tcaacactga ct 1782

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&lt;210&gt; SEQ ID NO 275

&lt;211&gt; LENGTH: 599

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
(LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 275

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20        25        30
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35        40        45
Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50        55        60
Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65        70        75        80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85        90        95
Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100       105       110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115       120       125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130       135       140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145       150       155       160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165       170       175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180       185       190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195       200       205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210       215       220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly

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225	230	235	240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met			
	245	250	255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His			
	260	265	270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val			
	275	280	285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr			
	290	295	300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly			
	305	310	315
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile			
	325	330	335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val			
	340	345	350
Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser			
	355	360	365
Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu			
	370	375	380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro			
	385	390	395
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val			
	405	410	415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met			
	420	425	430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser			
	435	440	445
Pro Gly Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly			
	450	455	460
Gly Gly Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu			
	465	470	475
Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn			
	485	490	495
Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met			
	500	505	510
Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu			
	515	520	525
Leu Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe			
	530	535	540
His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu			
	545	550	555
Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu			
	565	570	575
Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln			
	580	585	590
Ser Ile Ile Ser Thr Leu Thr			
	595		

&lt;210&gt; SEQ ID NO 276

&lt;211&gt; LENGTH: 1797

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

 <223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
 (LALA P329G)-IL-2 qm

-continued

&lt;400&gt; SEQUENCE: 276

```

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg    60
tcctgcaagg ccagcggtcta caccctcacc gagttcgga tgaactgggt ccgacaggct    120
ccagggcagg gcctcgaaatg gatgggctgg atcaacacca agaccggcga ggccacctac    180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac    240
atggaactgc ggagcctgag aagcgacgac accgcccgtg actactgcgc cagatgggac    300
ttcgctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct    360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc    420
ggcggaacag ccgccctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg    480
tcctggaaca gcggagccct gaccagcggc gtgcacacct ttccagcgt gctgcagagc    540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcaccag    600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag    660
cccaagagct gcgacaagac ccacacctgt ccccttgttc ctgcccctga gctgctgggc    720
ggacccagcg tgttcctgtt ccccccaaag cccaaggaca ccctgatgat cagccggacc    780
cccgaaagtg cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagtccaat    840
tgggtacgtg acggcgtgga ggtgcacaat gccaaagacca agccccggga ggaacagtac    900
aacagcacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc    960
aaagagtaca agtgcaaggc ctccaacaag gccctgcctg ccccatcgga gaaaaccatc   1020
agcaaggcca agggccagcc cagagaaccc caggtgtaca ccctgcccc ctgcagagat   1080
gagctgacca agaaccaggc gtccctgtgg tgtctggta agggcttcta cccagcgat   1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct   1200
gtgctggaca gcgacggcag cttcttctg tactccaaac tgaccgtgga caagagccgg   1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggcctgca caaccactac   1320
acccagaagt ccctgagcct gagccccggc aagtcggag gcggaggctc cggcgcgga   1380
ggttctggcg gaggtggcgc tctgcctcc tccagcacca agaaaacca gctccagctg   1440
gaacatctcc tgctggatct gcagatgatc ctgaacggca tcaacaacta caagaacccc   1500
aagctgaccc ggatgctgac cgccaagttc gccatgcccc agaaggccac cgagctgaaa   1560
catctgcagt gcctggaaga ggaactgaag cctctggaag aggtgctgaa cggcgcccag   1620
tccaagaact tccacctgag gcctcgggac ctgatctcca acatcaacgt gatcgtgctg   1680
gaactgaagg gctccgagac aaccttcattg tgcgagtacg ccgacgagac agctaccatc   1740
gtggaatttc tgaaccggtg gatcaccttc gccagtgcca tcattctccac cctgacc   1797

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&lt;210&gt; SEQ ID NO 277

&lt;211&gt; LENGTH: 598

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
(LALA P329G)-IL-2 qm (2)

&lt;400&gt; SEQUENCE: 277

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1           5           10           15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20           25           30

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Gly	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
	35						40					45			
Gly	Trp	Ile	Asn	Thr	Lys	Thr	Gly	Glu	Ala	Thr	Tyr	Val	Glu	Glu	Phe
	50					55					60				
Lys	Gly	Arg	Val	Thr	Phe	Thr	Thr	Asp	Thr	Ser	Thr	Ser	Thr	Ala	Tyr
	65				70					75					80
Met	Glu	Leu	Arg	Ser	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85					90						95	
Ala	Arg	Trp	Asp	Phe	Ala	Tyr	Tyr	Val	Glu	Ala	Met	Asp	Tyr	Trp	Gly
			100					105						110	
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser
		115					120						125		
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
	130					135					140				
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
	145				150					155					160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala
				165					170					175	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
		180						185					190		
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His
		195					200					205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys
	210					215					220				
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly
	225				230					235					240
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
				245					250					255	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		260						265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
	305				310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile
				325					330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		340						345					350		
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
		355					360					365			
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
	385				390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
				405					410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
	435						440					445			

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Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 450 455 460

Ser Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu  
 465 470 475 480

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr  
 485 490 495

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro  
 500 505 510

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu  
 515 520 525

Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His  
 530 535 540

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu  
 545 550 555 560

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr  
 565 570 575

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser  
 580 585 590

Ile Ile Ser Thr Leu Thr  
 595

<210> SEQ ID NO 278  
 <211> LENGTH: 1794  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
 (LALA P329G)-IL-2 qm (2)

<400> SEQUENCE: 278

caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaaggtg	60
tctctcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct	120
ccaggccagg gcctcgaatg gatgggtgg atcaacacca agaccggcga ggccacctac	180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac	240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac	300
ttcgctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct	360
agcgctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct	420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg	480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc	540
tcaggactct actccctcag cagcgtgggt accgtgccct ccagcagctt gggcacccag	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaactct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg	720
ggaccgtcag tcttcctctt ccccccaaaa cccaaggaca ccctcatgat ctcccgacc	780
cctgaggtea catgcgtggt ggtggacgtg agccacgaag accctgaggt caagtccaac	840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac	900
aacagcacgt accgtgtggt cagcgtcttc accgtcctgc accaggactg gctgaatggc	960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc	1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atgccgggat	1080
gagctgacca agaaccaggt cagcctgtgg tgccctggtea aaggcttcta tcccagcgac	1140

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atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc 1200
gtgctggact ccgacggctc cttcttcttc tacagcaagc tcaccgtgga caagagcagg 1260
tggcagcagg ggaacgtctt ctcattgctcc gtgatgcatg aggctctgca caaccactac 1320
acgcagaaga gcctctccct gtctccgggt ggccggcgag gctccggagg cggaggttct 1380
ggcggagggt gctccgcacc tgctcaagt tctacaaaga aaacacagct acaactggag 1440
catttactgc tggatttaca gatgattttg aatggaatta ataattacaa gaatcccaaa 1500
ctcaccagga tgctcacagc caagtttgcc atgcccaga aggccacaga actgaaacat 1560
cttcagtgtc tagaagaaga actcaaacct ctggaggaag tgctaaatgg cgctcaaagc 1620
aaaaactttc acttaagacc cagggactta atcagcaata tcaacgtaat agttctggaa 1680
ctaaagggat ctgaaacaac attcatgtgt gaatatgctg atgagacagc aaccattgta 1740
gaatttctga acagatggat tacctttgcc caaagcatca tctcaacact gact 1794

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&lt;210&gt; SEQ ID NO 279

&lt;211&gt; LENGTH: 598

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
(LALA P329G) -IL-2 wt

&lt;400&gt; SEQUENCE: 279

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20        25        30
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35        40        45
Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50        55        60
Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65        70        75        80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85        90        95
Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100       105       110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115       120       125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130       135       140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145       150       155       160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165       170       175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180       185       190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195       200       205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210       215       220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225       230       235       240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met

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245					250					255					
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
			260					265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
305					310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile
			325						330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		340						345					350		
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
		355					360					365			
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
385					390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
			405						410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
		435					440					445			
Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	450					455					460				
Ser	Ala	Pro	Thr	Ser	Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu
465					470					475					480
His	Leu	Leu	Leu	Asp	Leu	Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr
			485					490						495	
Lys	Asn	Pro	Lys	Leu	Thr	Arg	Met	Leu	Thr	Phe	Lys	Phe	Tyr	Met	Pro
		500						505					510		
Lys	Lys	Ala	Thr	Glu	Leu	Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu
		515					520					525			
Lys	Pro	Leu	Glu	Glu	Val	Leu	Asn	Leu	Ala	Gln	Ser	Lys	Asn	Phe	His
	530					535					540				
Leu	Arg	Pro	Arg	Asp	Leu	Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu
545					550					555					560
Leu	Lys	Gly	Ser	Glu	Thr	Thr	Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr
			565					570						575	
Ala	Thr	Ile	Val	Glu	Phe	Leu	Asn	Arg	Trp	Ile	Thr	Phe	Ala	Gln	Ser
			580					585						590	
Ile	Ile	Ser	Thr	Leu	Thr										
		595													

&lt;210&gt; SEQ ID NO 280

&lt;211&gt; LENGTH: 1794

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc knob  
(LALA P329G) - IL-2 wt

&lt;400&gt; SEQUENCE: 280

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cagggtgcagc	tggtgcagtc	tgccgcccga	gtgaagaaac	ctggagctag	tgtgaagggtg	60
tcctgcaagg	ccagcggtta	caccttcacc	gagttcgga	tgaactgggt	ccgacaggct	120
ccagggcagg	gcctcgaaatg	gatgggctgg	atcaaacacca	agaccggcga	ggccacctac	180
gtggaagagt	tcaagggcag	agtgaccttc	accacggaca	ccagcaccag	caccgcctac	240
atggaactgc	ggagcctgag	aagcgacgac	accgcccgtg	actactgcgc	cagatgggac	300
ttcgctatt	acgtggaagc	catggactac	tggggccagg	gcaccaccgt	gaccgtgtct	360
agcgctagca	ccaagggccc	atcggtcttc	cccctggcac	cctcctccaa	gagcacctct	420
gggggacacag	cggccctggg	ctgcctggtc	aaggactact	tcccgaacc	ggtgacgggtg	480
tcgtggaact	caggcgccct	gaccagcggc	gtgcacacct	tcccggtgtg	cctacagtcc	540
tcaggactct	actccctcag	cagcgtgggt	accgtgccct	ccagcagctt	gggcaccacg	600
acctacatct	gcaacgtgaa	tcacaagccc	agcaaacacca	aggtggacaa	gaaagttag	660
cccaaattctt	gtgacaaaac	tcacacatgc	ccaccgtgcc	cagcacctga	agctgcaggg	720
ggaccgtcag	tcttctctct	ccccccaaaa	cccaaggaca	ccctcatgat	ctcccggacc	780
cctgagggtca	catgcgtggg	ggtggacgtg	agccacgaag	accctgaggt	caagttcaac	840
tggtacgtgg	acggcgtgga	ggtgcataat	gccaaagaaa	agccgcggga	ggagcagtac	900
aacagcacgt	accgtgtggg	cagcgtcttc	accgtctctg	accaggactg	gctgaatggc	960
aaggagtaca	agtgcagggt	ctccaacaaa	gccctcggcg	cccccatcga	gaaaaccatc	1020
tccaaagcca	aagggcagcc	ccgagaacca	caggtgtaca	ccctgcccc	atgccgggat	1080
gagctgacca	agaaccagggt	cagcctgtgg	tgcttggtca	aaggtctcta	tcccagcgac	1140
atcgccgtgg	agtgaggagag	caatgggcag	ccggagaaca	actacaagac	cacgcctccc	1200
gtgctggact	ccgacggctc	cttcttcttc	tacagcaagc	tcaccgtgga	caagagcagg	1260
tggcagcagg	ggaacgtctt	ctcatgctcc	gtgatgcatg	aggctctgca	caaccactac	1320
acgcagaaga	gcctctccct	gtctccgggt	ggcggcggag	gctccggagg	cggaggttct	1380
ggaggcggag	gctccgcacc	tacttcaagt	tctacaaaga	aaacacagct	acaactggag	1440
catttactgc	tggatttaca	gatgattttg	aatggaatta	ataattacaa	gaatcccaaa	1500
ctcaccagga	tgctcacatt	taagttttac	atgcccaaga	aggccacaga	actgaaacat	1560
cttcagtgtc	tagaagaaga	actcaaacct	ctggaggaag	tgctaaattt	agctcaaagc	1620
aaaaactttc	acttaagacc	cagggactta	atcagcaata	tcaacgtaat	agttctggaa	1680
ctaaagggat	ctgaacaac	attcatgtgt	gaatatgctg	atgagacagc	aaccattgta	1740
gaattttctga	acagatggat	tacctttgcc	caaagcatca	tctcaacact	gact	1794

&lt;210&gt; SEQ ID NO 281

&lt;211&gt; LENGTH: 451

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 281

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

[illegible]

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<210> SEQ ID NO 282  
 <211> LENGTH: 1353  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab HC-Fc hole (LALA P329G)

<400> SEQUENCE: 282

```

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaaggtg      60
tcctgcaagg ccagcggtcta caccttcacc gagttcgga tgaactgggt ccgacaggct      120
ccagggcagg gcctcgaaatg gatgggctgg atcaacacca agaccggcga ggccacctac      180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac      240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac      300
ttcgctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct      360
agcgctagca ccaagggccc ctccgtgttc cccctggccc ccagcagcaa gagcaccagc      420
ggcggcacag ccgctctggg ctgcctggtc aaggactact tccccgagcc cgtgaccgtg      480
tcctggaaca gcggagccct gacctccggc gtgcacacct tccccgccgt gctgcagagt      540
tctggcctgt atagcctgag cagcgtggtc accgtgcctt ctageagcct gggcaccacg      600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaagtgaggag      660
cccaagagct gcgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttctcttt cccccaaaaa cccaaggaca ccctcatgat ctcccgacc      780
cctgaggtea catgcgtggt ggtggacgtg agccacgaag accctgagggt caagtccaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac      900
aacagcacgt accgtgtggt cagcgtcttc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaagggt ctccaacaaa gccctcgcg ccccatcga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtgca ccctgcccc atcccgggat     1080
gagctgacca agaaccagggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttcttctc gtgagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggtcttgca caaccactac     1320
acgcagaaga gcctctccct gtctccgggt aaa                                     1353

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<210> SEQ ID NO 283  
 <211> LENGTH: 215  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1 Fab LC

<400> SEQUENCE: 283

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
20          25          30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35          40          45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
50          55          60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65          70          75          80

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Gly	Gly	Ile	Ile	Pro	Ile	Phe	Gly	Thr	Ala	Asn	Tyr	Ala	Gln	Lys	Phe
50						55					60				
Gln	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Leu	Tyr	Gly	Tyr	Ala	Tyr	Tyr	Gly	Ala	Phe	Asp	Tyr	Trp	Gly
			100					105					110		
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser
		115					120					125			
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
	130					135					140				
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
145					150					155					160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala
				165					170					175	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
		180						185					190		
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His
		195					200					205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys
	210					215					220				
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly
225					230					235					240
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
				245					250					255	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
			260					265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
305					310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile
				325					330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
			340					345					350		
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
		355					360					365			
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
385					390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
				405					410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
		435					440					445			
Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	450					455					460				
Ser	Ala	Pro	Ala	Ser	Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu

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465	470	475	480
His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr			
	485	490	495
Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro			
	500	505	510
Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu			
	515	520	525
Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His			
	530	535	540
Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu			
	545	550	555
Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr			
	565	570	575
Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser			
	580	585	590
Ile Ile Ser Thr Leu Thr			
	595		

&lt;210&gt; SEQ ID NO 286

&lt;211&gt; LENGTH: 1794

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 286

cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc	60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc	120
cctggacaag ggctgcagtg gatgggaggg atcatcccta tctttggtac agcaaaactac	180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac	240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac	300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc	360
tcagctagca ccaagggccc atcgggtctt cccctggcac cctcctccaa gagcacctct	420
gggggacacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg	480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggtgtg cctacagtcc	540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggaaccacg	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg	720
ggaccgtcag tcttcctctt cccccaaaa cccaaggaca ccctcatgat ctcccggacc	780
cctgaggtea catgctgggt ggtggacgtg agccacgaag accctgaggt caagttcaac	840
tggtagctgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtag	900
aacagcacgt accgtgtggt cagcgtcttc accgtcctgc accaggactg gctgaatggc	960
aaggagtaca agtgcaaggc ctccaacaaa gccctcggcg ccccatcgga gaaaaccatc	1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atgccgggat	1080
gagctgacca agaaccaggt cagcctgtgg tgcctggtea aaggcttcta tcccagcgac	1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc	1200
gtgctggact ccgacggctc cttcttcttc tacagcaagc tcaccgtgga caagagcagg	1260
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggtctctga caaccactac	1320

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acgcagaaga gcctctccct gtctccgggt gccggcggag gctccggagg cggaggttct 1380
ggcggagggtg gctccgcacc tgctcaagt tctacaaaga aaacacagct acaactggag 1440
catttactgc tggatttaca gatgattttg aatggaatta ataattacaa gaatcccaaa 1500
ctcaccagga tgctcacagc caagtttgcc atgcccaaga aggccacaga actgaaacat 1560
cttcagtgtc tagaagaaga actcaaacct ctggaggaag tgctaaatgg cgctcaaagc 1620
aaaaactttc acttaagacc cagggactta atcagcaata tcaacgtaat agttctggaa 1680
ctaaagggat ctgaacaac attcatgtgt gaatatgctg atgagacagc aaccattgta 1740
gaatttctga acagatggat tacctttgcc caaagcatca tctcaacact gact 1794

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&lt;210&gt; SEQ ID NO 287

&lt;211&gt; LENGTH: 451

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 287

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1           5           10           15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20           25           30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35           40           45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50           55           60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85           90           95
Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100          105          110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115          120          125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130          135          140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145          150          155          160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165          170          175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180          185          190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195          200          205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210          215          220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225          230          235          240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245          250          255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260          265          270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val

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275	280	285
His Asn Ala Lys Thr Lys	Pro Arg Glu Glu Gln Tyr	Asn Ser Thr Tyr
290	295	300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly		
305	310	315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile		
	325	330 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val		
	340	345 350
Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser		
	355	360 365
Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu		
	370	375 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro		
	385	390 395 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val		
	405	410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met		
	420	425 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser		
	435	440 445
Pro Gly Lys		
450		

&lt;210&gt; SEQ ID NO 288

&lt;211&gt; LENGTH: 1353

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: 2B10 Fab HC-Fc hole (LALA P329G)

&lt;400&gt; SEQUENCE: 288

```

cagggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tcagctagca ccaagggccc ctccgtgttc cccctggccc ccagcagcaa gagcaccagc      420
ggcggcacag ccgctctggg ctgcctggtc aaggactact tccccgagcc cgtgaccgtg      480
tcctggaaca gcggagccct gacctccggc gtgcacacct tccccgccgt gctgcagagt      540
tctggcctgt atagcctgag cagcgtggtc accgtgcctt ctagcagcct gggaaccag      600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag      660
cccaagagct gcgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttctcttt cccccaaaaa cccaaggaca ccctcatgat ctcccggacc      780
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttaaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac      900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaagggt ctccaacaaa gccctcggcg ccccatcgga gaaaaccatc     1020

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tccaaagcca aagggcagcc ccgagaacca caggtgtgca cctgcccc atcccgggat 1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac 1140
atcgccgtgg agtgggagag caatgggcag cgggagaaca actacaagac cagcctccc 1200
gtgctggact ccgacggctc cttctctctc gtgagcaagc tcaccgtgga caagagcagg 1260
tggcagcagg ggaacgtctt ctcattgctc gtgatgcatg aggctctgca caaccactac 1320
acgcagaaga gcctctccct gtctccgggt aaa 1353

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<210> SEQ ID NO 289
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

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<400> SEQUENCE: 289

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```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100         105         110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115         120         125
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130         135         140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145         150         155         160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165         170         175
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180         185         190
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195         200         205
Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210         215         220
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu
225         230         235         240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245         250         255
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
260         265         270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275         280         285
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290         295         300

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Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys  
 305 310 315 320  
 Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys  
 325 330 335  
 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys  
 340 345 350  
 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys  
 355 360 365  
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
 370 375 380  
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly  
 385 390 395 400  
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
 405 410 415  
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
 420 425 430  
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly  
 435 440 445  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala Ser Ser  
 450 455 460  
 Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu  
 465 470 475 480  
 Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr  
 485 490 495  
 Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu  
 500 505 510  
 Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val  
 515 520 525  
 Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu  
 530 535 540  
 Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr  
 545 550 555 560  
 Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe  
 565 570 575  
 Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr  
 580 585 590

&lt;210&gt; SEQ ID NO 290

&lt;211&gt; LENGTH: 1776

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

&lt;400&gt; SEQUENCE: 290

```

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccgatt caccttagc agttatgcca tgagctgggt ccgccaggct      120
ccagggaagg ggtcggagt ggtctcagct attagtggta gtgtggttag cacatactac      180
gcgactccg tgaagggcgc gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc      300
ggatttgact actggggcca aggaacctg gtcaccgtct cgagtgctag caccaagggc      360
ccatcggtct tccccctggc accctcctcc aagagcacct ctgggggcac agcgccctg      420
ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgctgtggaa ctcaggcgcc      480

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ctgaccagcg gcgtgcacac ctccccggct gtcctacagt cctcaggact ctactccctc 540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg 600
aatcacaagc ccagcaacac caaggtggac aagaaagttg agcccaaadc ttgtgacaaa 660
actcacacat gcccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttctctc 720
ttccccccaa aacccaagga caccctcatg atctcccgga cccctgaggt cacatgcgtg 780
gtggtggacg tgagccacga agaccctgag gtcaagtcca actgggtacgt ggacggcgtg 840
gaggtgcata atgccaagac aaagccgagg gaggagcagt acaacagcac gtaccgtgtg 900
gtcagcgtcc tcaccgtctc gcaccaggac tggctgaatg gcaaggagta caagtgcaag 960
gtctccaaca aagccctcgg cgcccccatc gagaaaacca tctccaaagc caaagggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag 1080
gtcagcctgt ggtgcctggt caaaggcttc tatcccagcg acatgccgtt ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc 1200
tccttctctc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtggcggcgg aggctccgga ggcggaggtt ctggcggagg tggctccgca 1380
cctgcctcaa gttctacaaa gaaaacacag ctacaactgg agcatttact gctggattta 1440
cagatgattt tgaatggaat taataattac aagaatccca aactcaccag gatgctcaca 1500
gccaagtgtt ccatgcccaa gaaggccaca gaactgaaac atcttcagtg tctagaagaa 1560
gaactcaaac ctctggagga agtgcataat ggcgctcaaa gcaaaaactt tcacttaaga 1620
cccagggact taatcagcaa tatcaacgta atagttctgg aactaaaggg atctgaaaca 1680
acattcatgt gtgaatatgc tgatgagaca gcaaccattg tagaatttct gaacagatgg 1740
attacctttg cccaaagcat catctcaaca ctgact 1776

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&lt;210&gt; SEQ ID NO 291

&lt;211&gt; LENGTH: 592

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 wt (2)

&lt;400&gt; SEQUENCE: 291

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100         105         110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115         120         125

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Ser 130	Lys	Ser	Thr	Thr	Ser	Gly 135	Gly	Thr	Ala	Ala	Leu 140	Gly	Cys	Leu	Val
Lys 145	Asp	Tyr	Phe	Pro	Glu 150	Pro	Val	Thr	Val	Ser 155	Trp	Asn	Ser	Gly	Ala 160
Leu	Thr	Ser	Gly	Val	His 165	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly 175
Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly 190
Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys 205
Val	Asp	Lys	Lys	Val	Glu 210	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys 220
Pro	Pro	Cys	Pro	Ala	Pro 225	Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val	Phe	Leu 230
Phe	Pro	Pro	Lys	Pro	Lys 235	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu 240
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys 245
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys 250
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu 255
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys 260
Val	Ser	Asn	Lys	Ala	Leu	Gly	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys 265
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Cys 270
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys	Leu	Val	Lys 275
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln 280
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly 285
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln 290
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn 295
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly 300
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Pro	Thr	Ser	Ser 305
Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	Leu 310
Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	Thr 315
Arg	Met	Leu	Thr	Phe	Lys	Phe	Tyr	Met	Pro	Lys	Lys	Ala	Thr	Glu	Leu 320
Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val 325
Leu	Asn	Leu	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	Leu 330
Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	Thr 335

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545	550	555	560
Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe			
	565	570	575
Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr			
	580	585	590

<210> SEQ ID NO 292  
 <211> LENGTH: 1776  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 wt (2)

<400> SEQUENCE: 292

gagggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc	60
tcctgtgcag cctccggatt caccttagc agttatgcc tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc	300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc	360
ccatcggtct tccccctggc accctcctcc aagagcacct ctgggggcac agcggccctg	420
ggctgcctgg tcaaggacta ctccccgaa ccggtgacgg tgctcgtgga ctcaggcgcc	480
ctgaccagcg gcgtgcacac ctccccggt gtcctacagt cctcaggact ctactccctc	540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg	600
aatcacaagc ccagcaacac caaggtggac aagaaagtgt agcccaaatac ttgtgacaaa	660
actcacacat gccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttctctc	720
ttcccccaa aacccaagga caccctcatg atctcccgga cccctgaggt cacatgcgtg	780
gtggtggacg tgagccacga agaccctgag gtcaagtcca actgggtacgt ggacggcgtg	840
gagggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg	900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcaag	960
gtctccaaca aagccctcgg cgccccatc gagaaaacca tctccaaagc caaagggcag	1020
ccccgagaac cacaggtgta caccctgcc ccatgccggg atgagctgac caagaaccag	1080
gtcagcctgt ggtgcctggt caaaggcttc tatcccagcg acatgcctgt ggagtgggag	1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc	1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc	1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc	1320
ctgtctccgg gtggcgcgcg aggctccgga ggcggaggtt ctggaggcgg aggctccgca	1380
cctacttcaa gttctacaaa gaaaacacag ctacaactgg agcatcttact gctggattta	1440
cagatgattt tgaatggaat taataattac aagaatccca aactcaccag gatgctcaca	1500
tttaagtgtt acatgcccac gaaggccaca gaactgaaac atcttcagtg tctagaagaa	1560
gaactcaaac ctctggagga agtgctaaat ttagctcaaa gcaaaaactt tcacttaaga	1620
cccagggact taatcagcaa tatcaacgta atagttctgg aactaaaggg atctgaaaca	1680
acattcatgt gtgaatatgc tgatgagaca gcaaccattg tagaatttct gaacagatgg	1740
attacctttg cccaaagcat catctcaaca ctgact	1776

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<210> SEQ ID NO 293  
 <211> LENGTH: 108  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1; VL

<400> SEQUENCE: 293

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr  
 20 25 30  
 Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45  
 Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu  
 85 90 95  
 Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> SEQ ID NO 294  
 <211> LENGTH: 324  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1; VL

<400> SEQUENCE: 294

gatatccaga tgaccagtc tccatctcc ctgtctgcat ctgtgggaga cagagtcacc 60  
 atcacttgca aggccagtgc ggctgtgggt acgtatgttg cgtgggtatca gcagaaacca 120  
 gggaaagcac ctaagctcct gatctattcg gcactctacc gcaaaagggg agtcccatca 180  
 aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240  
 gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc 300  
 cagggcacca agctcgagat caag 324

<210> SEQ ID NO 295  
 <211> LENGTH: 121  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: CH1A1A 98/99 2F1; VH

<400> SEQUENCE: 295

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
 20 25 30  
 Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe  
 50 55 60  
 Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

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Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly  
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> SEQ ID NO 296

<211> LENGTH: 363

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CH1A1A 98/99 2F1; VH

<400> SEQUENCE: 296

cagggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaaggtg 60  
 tcttgcaagg ccagcggcta caccctcacc gagttcggca tgaactgggt ccgacaggct 120  
 ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180  
 gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac 240  
 atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac 300  
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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DP47GS VL

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 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro  
 85 90 95

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
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<211> LENGTH: 324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DP47GS VL

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 cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180  
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240

-continued

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caggggacca aagtggaaat caaa 324
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20          25          30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100         105         110
Val Ser Ser
115
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ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
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ggatttgact actggggcca aggaaccctg gtcaccgtct cgagt 345
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The invention claimed is:

1. An immunoconjugate comprising a first and a second antigen binding moiety, and an Fc domain consisting of two subunits, and an effector moiety, wherein the effector moiety is a cytokine, wherein not more than one effector moiety is present, and further wherein said Fc domain comprises a modification promoting heterodimerization of two non-identical polypeptide chains.

2. The immunoconjugate of claim 1, wherein said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain.

3. The immunoconjugate of claim 2, wherein said effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification.

4. The immunoconjugate of claim 2, wherein said knob modification comprises the amino acid substitution T366W, and said hole modification comprises the amino acid substitutions T366S, L368A and Y407V, according to the EU numbering system as described in Kabat.

5. The immunoconjugate of claim 1, wherein said Fc domain is engineered to have altered binding to an Fc receptor and/or altered effector function.

6. The immunoconjugate of claim 5, wherein said Fc receptor is an Fcγ receptor.

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7. The immunoconjugate of claim 5, wherein said effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

8. The immunoconjugate of claim 5, wherein said altered binding and/or effector function is reduced binding and/or effector function.

9. The immunoconjugate of claim 8, wherein said Fc domain comprises one or more amino acid mutations that reduce the binding of the Fc domain to an Fc receptor.

10. The immunoconjugate of claim 9, wherein said amino acid mutation is an amino acid substitution at position P329, according to the EU numbering system as described in Kabat.

11. The immunoconjugate of claim 9, wherein the Fc domain comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits, according to the EU numbering system as described in Kabat.

12. The immunoconjugate of claim 9, wherein said Fc receptor is an Fcγ receptor.

13. The immunoconjugate of claim 1, wherein said cytokine is IL-2.

14. A pharmaceutical composition comprising the immunoconjugate of claim 1 and a pharmaceutically acceptable carrier.

15. The immunoconjugate of claim 1, wherein said effector moiety is fused to the amino- or carboxy-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide.

16. The immunoconjugate of claim 1, wherein said first and second antigen binding moieties are each fused to the amino-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide or an immunoglobulin hinge region.

17. The immunoconjugate of claim 1, wherein said first and second antigen binding moieties are each a Fab molecule.

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18. The immunoconjugate of claim 1, wherein said IgG Fc domain is an IgG1 Fc domain.

19. The immunoconjugate of claim 18, wherein said Fc domain is an IgG Fc domain.

20. The immunoconjugate of claim 1, wherein said cytokine is a mutant IL-2 polypeptide having reduced binding affinity to the α-subunit of the IL-2 receptor.

21. The immunoconjugate of claim 20, wherein said mutant IL-2 polypeptide comprises an amino acid substitution at one or more positions selected from the positions corresponding to residues 42, 45 and 72 of human IL-2.

22. The immunoconjugate of claim 21, wherein said first and a second antigen binding moieties are Fab molecules directed to CEA and each comprise a heavy chain variable region sequence of SEQ ID NO: 191, and a light chain variable region sequence of SEQ ID NO: 189; and wherein said mutant IL-2 polypeptide comprises the sequence of SEQ ID NO: 3.

23. The immunoconjugate of claim 22, wherein the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 277, SEQ ID NO: 281 and SEQ ID NO: 283.

24. The immunoconjugate of claim 1, wherein said first and said second antigen binding moiety and said Fc domain are part of an immunoglobulin molecule.

25. The immunoconjugate of claim 24, wherein said immunoglobulin molecule is an IgG class immunoglobulin.

26. The immunoconjugate of claim 25, wherein said IgG class immunoglobulin is an IgG1 subclass immunoglobulin.

27. The immunoconjugate of claim 24, wherein said effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

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